

Effects of Nicotine and Stress on Anxiety-related and Depression-related  
Behavior in Rats

by

Matthew J. Moosey

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## **DEDICATIONS**

This project is dedicated to the memory of the fallen Soldiers of the 1<sup>st</sup> Battalion,  
22<sup>nd</sup> Infantry Regiment.

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UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES  
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FAX: 301-295-6772

Title of Thesis: " Effects of Nicotine and Stress on Anxiety-related  
and Depression-related Behavior in Rats"

Name of Candidate: Matthew J. Moosey  
Clinical Psychology  
Master of Science  
July 29, 2014

Thesis and Abstract Approved:

Neil E. Grunberg, Ph.D.  
Department of Medical and Clinical Psychology  
Thesis Advisor

7/29/14  
Date

Andrew Waters, Ph.D.  
Department of Medical and Clinical Psychology  
Committee Member

7/30/14  
Date

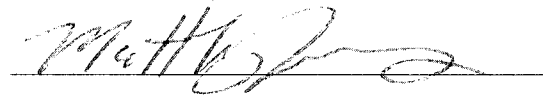
Jeanette Witter, Ph.D.  
Department of Medical and Clinical Psychology  
Committee Member

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Matthew J. Moosey

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## **ABSTRACT**

Title of Thesis: Effects of Nicotine and Stress on Anxiety-related and Depression-related Behavior in Rats

Matthew J. Moosey, Master of Science, 2014

Thesis directed by: Neil E. Grunberg, Professor, MPS

Current cigarette use in the U.S. includes roughly 20% of civilian adults, 30% of Armed Forces personnel, and a majority of psychiatric patients. Tobacco use by American Warriors deployed to Iraq and Afghanistan is estimated at approximately 50%, despite widespread knowledge of the health risks associated with tobacco use. Two animal (rat) experiments were conducted to examine whether nicotine, the drug of addiction in tobacco, decreases anxiety-related and depression-related behavior. In Experiment 1, rats (male and female, Sprague-Dawley rats) were exposed to the Warrior Stress Paradigm (a paradigm that mimics the threat of death and environmental stressors experienced by Warriors in combat) and nicotine was delivered via SC implanted osmotic minipumps at three levels of nicotine dosages (0, 3, and 6 mg nic/kg/day). Planned comparisons between saline controls and unstressed female rats in the 6 mg nic/kg/day nicotine condition demonstrated less depression-related behavior at certain time points, yet these findings for females (and not males) were preliminary and warranted replication. There were no anxiolytic effects of nicotine in the first experiment. A second experiment used four dosages of nicotine (0, 3, 6, 9 mg nic/kg/day) and failed to find clear anti-depressant effects of nicotine. Future studies could use varying levels of

Warrior stress or different predatory stressors to investigate how sex may moderate the anxiolytic or anti-depressant properties of nicotine, which may inform tobacco cessation treatment in male and female Warriors.

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## CHAPTER 1: Introduction

In the fall of 1988, two major events in American public health occurred. The first was the release of *The Health Consequences of Smoking: Nicotine Addiction: A Report of the Surgeon General*, which marked the first time that the Federal government definitively concluded that nicotine within tobacco is an addictive substance and that tobacco use should be treated as an addiction. The second related event was the initiation of an ambitious project designed to achieve a tobacco-free society by educating very young children about tobacco and nicotine addiction. Across the country, eager 7 year olds (including the author of this paper) started first grade with the high hopes of educators, scientists, and health professionals that these children would be the “Smoke Free Class of 2000.” A joint-initiative of multiple government agencies, the American Heart Association, American Lung Association, and American Cancer Society, the Smoke Free Class of 2000 (SFC 2000) was a program designed to create a smoke free society by educating children from the earliest possible years in school about the health risks associated with tobacco use. The program distributed a wide range of smoking prevention materials, including t-shirts, coloring books, and even catchy classroom jingles. The most visible part of this campaign included encouragement from the legendary U.S. Surgeon General C. Everett Koop, who was among the first prominent health care professionals to confront the politically powerful tobacco lobby. Encouraged by Dr. Koop’s determination to make a smoke-free society, SFC 2000 educated young children about the health dangers associated with tobacco use and it was among the first public awareness campaigns to highlight the deceptive practices of tobacco advertising and the dangers of second-hand (“involuntary”) smoke (8). Millions of dollars were

invested into a program that targeted approximately 80,000 first grade classes across the United States (24). Despite the best intentions and earnest efforts of SFC 2000, the project did not achieve its goals. Although anti-tobacco programs have helped to decrease tobacco use in the U.S., in 2000 nearly 30% of Americans aged 18-24 used cigarettes (12). A generation that was raised to understand the substantial health risks of tobacco use from the earliest possible age still smoked. This paradox raises some troubling questions- were children smoking to “be rebellious” or “because it looks ‘cool’” or was there something about the psychobiological properties of tobacco that continued to make it an irresistible drug despite this generation’s extensive knowledge about the dangers of tobacco use?

### **Nicotine**

It is well established in scientific literature that nicotine is a highly addictive naturally occurring substance found in large quantities within the tobacco leaf (13; 42; 56; 59; 66). Furthermore, investigations have revealed that nicotine self-administration is reinforced by other “positive” effects including increased attention, altered reaction time, and attenuated negative affect (4; 23; 33; 61). Additionally, numerous studies suggest that the desirable psychoactive effects of nicotine contribute to long-term tobacco use and nicotine dependency (14; 25; 27; 31; 32; 34; 55; 65). Moreover, a large body of epidemiological studies indicate that depressed humans often use tobacco products, and it has been suggested that tobacco use may help to modulate mood and control depression (6; 7; 16; 44; 47; 63). These studies, however, are largely limited to self-report measures, have significant individual differences among smokers, and because of ethical considerations, lack a true control (19; 26). Because of these limitations, several studies

have examined the potential anti-depressant effects of nicotine in animal models (50; 57) and have reported that nicotine decreases depression-related behavior. Findings concerning the anxiolytic properties of nicotine in humans and animals have been mixed (59). Some studies have suggested that the “calming effect” that smokers experience is unlike established anxiolytic drugs like diazepam, but rather a result of activation of mesolimbic dopamine (3). Picciotto et al (46) highlight the ambiguity of nicotine’s relationship with stress suggesting that the drug can either increase or decrease anxiety-related behavior depending on the animal model and environmental conditions used during administration. These studies are limited to acute administration of nicotine through subcutaneous (SC) injections which has limitations including several injections daily as one way to model human smoking behavior (15). Additionally, these studies often lack female subjects (45) which limits generalizability of findings.

### **Nicotine Use in Combat**

The complex relationship among the stress response, depression, anxiety, and nicotine is especially salient in modern combat environments where combat-related stress-disorders (e.g., post-traumatic stress disorder [PTSD]) are often comorbid with nicotine use and dependency (52). The military has identified tobacco use and related health risks to be a significant threat to unit and mission readiness, spending significant resources on smoking cessation, raising public awareness to the risks of tobacco use, and enacting policies designed to discourage tobacco use. Despite these efforts, use of tobacco among Warriors continues to grow at an alarming rate, especially among Millennials (Americans born between 1982-1994) who regularly smoked cigarettes while deployed to Operation Iraqi Freedom. Brown (9) reported that **nearly 40% of male**

**combat veterans and 44% of female combat veterans born between the years of 1985-1989 regularly use tobacco products.** Could tobacco use among young Warriors (especially females) be self-medicating under stress using tobacco products for anxiolytic and anti-depressant properties of nicotine?

### **Specific Aims of Present Study**

In addition to the limitations of using self-report data for general tobacco use among humans, the logistical challenges of conducting research on Warriors in combat are numerous and underscore the utility of an animal model. The research project presented examines some of the pressing questions that remain about tobacco use among Warriors using an animal model, a model of Warrior stress, and a minimally invasive nicotine delivery system.

The specific aims of the project were:

1. To examine effects of nicotine administered under stressful and non-stressful conditions on depression-related and anxiety-related behaviors.
2. To determine whether there are sex differences in depression-related and anxiety-related behavior in response to stress and nicotine.

## **CHAPTER 2: Overview and Hypotheses – Experiment 1**

The purpose of this experiment was to investigate the effect of sustained nicotine administration and environmental stress on anxiety-related and depression-related behavior of male and female Sprague Dawley young adult rats. The first experiment used a 2 (Male, Female) x 3 (0, 3, 6 mg nic/kg/day) x 2 (no stress, stress) full-factorial design that yielded 12 experimental conditions. The number of subjects in each experimental condition was eight, a number based on previous animal research examining similar variables to the current study (26; 31; 67). The experiment was divided into two phases: before and during nicotine or saline administration.

### **Hypotheses (H)**

**H1:** Stressed rats will show more anxiety-related and depression-related behavior than unstressed rats.

H1 Rationale: Previous literature (5; 67; 68) has demonstrated that exposure to the Warrior Stress model increases anxiety-related and depression-related behavior in male and female rats. This hypothesis ties to Aim 1.

**H2:** Nicotine will attenuate anxiety-related and depression-related behaviors based on the model and environment selected for the study.

H2 Rationale: Experiment 1 will grow the literature (3; 7; 23; 31; 60) addressing the positive effects of sustained nicotine use when exposed to environmental stress. This hypothesis ties to Aim 1.

**H3:** There will be a difference between anxiety-related and depression-related behavior between male and female rats; Males will show less anxiety-related and depression-related behavior than female rats.

H3 Rationale: Previous literature (5; 67; 68) has indicated that female rats are more sensitive to chronic predatory and environmental stress, thereby demonstrating more anxiety-related and depression-related behavior than male rats. This hypothesis ties to Aim 2.

**H4:** There will be a difference in the effects of nicotine between males and females rats.

H4 Rationale: Experiment 1 will grow the literature addressing sex-related differences in behavior between male and females. *A priori*, the direction of the differences is unknown. This hypothesis ties to Aim 2.

## **Timeline**

Upon arrival to the Laboratory of Animal Medicine (LAM) Facility, rats were gently handled by experimenters by stroking and petting the animals (gentling) and numbering the animals by marking the tails with indelible ink. The following day, rats were placed into the Open Field Apparatus (OFA) chambers for acclimation, with baseline (BL) OFA measured on day 3 (see page 8 for a detailed description). Additional OFA trials were measured on day 16 (Time 1 [T1]), day 23 (Time 2 [T2]), and day 29 (Time 3[T3]). On day 7, the animals in the stress condition were exposed to the first round of environmental and predatory stress (see page 44 for a detailed description). Stressing continued on every other day throughout the duration of the project to model environmental stress in combat. On day 14, the rats were anesthetized with 5% oxygen-

isoflurane and were surgically implanted with minipumps containing saline or varying dosages of nicotine bitartrate solution (see Appendix A for a detailed explanation of the nicotine solution calculations). Day 14 marked the completion of Phase 1 and the start of Phase 2. On day 32, the animals were anesthetized with carbon dioxide and sacrificed by decapitation. Trunk blood and brain tissue were extracted and frozen for future research projects. Appendix A includes a figure of the Experiment 1 timeline.

### **Subjects**

Animals used for Experiment 1 were 48 male and 48 female Sprague Dawley (SD) rats from Charles River Laboratories (Wilmington, Massachusetts). The rats were approximately 52 days old upon arrival which coincides developmentally with the period immediately following adolescence in SD rats (43). This age models the demographics of contemporary young adult Warriors currently deployed in support of the Global War on Terror (68). Both the strain and age of the animals selected in this study have been successfully used in several previous investigations of stress, nicotine, and traumatic brain injury (20; 21; 27; 28) - topics that are especially pertinent to modern-day Warriors. Because sex-related differences were a major interest of the study, male and female rats were included.

## **CHAPTER 3: Methods- Experiment 1**

### **Phase 1: Pre-implant**

Phase one lasted for 14 days after the arrival of the animals. Immediately after arriving at the Laboratory of Animal Medicine (LAM) facility, each animal was gently held, petted, for several minutes to accustom the animal to being handled by human experimenters. Additionally, each animal's tail was marked with a numbering convention designed to easily verify the subject's identification number. Following gentling and numbering, animals were randomly assigned to stress conditions, with assignment to drug condition semi-randomly assigned by grouping animals of similar weight together. The following day, animals were gentled again before being placed into the OFA chambers to acclimatize the animal prior to taking baseline (BL) measurements on day 3. For locomotor activity measurement, animals were placed into the OFA chambers and left unperturbed in a darkened environment for 60 minutes before being returned to their home cages. On day 7, animals in the stress condition were exposed to their first round of environmental and predatory stress. Environmental and predatory stressing was conducted every other day throughout the course of the experiment. Body weight was measured weekly using an electronic laboratory balance that takes multiple weights to control for movement artifacts. Body weight was used as an indirect measure that the nicotine-containing minipumps were functioning because nicotine affects animal body weight (26; 28; 31; 32).

To model the continuous nicotine use of male and female Warriors in environmentally stressful conditions, osmotic minipumps (Alzet Model 2002, Durect Corporation) were filled with different dosages of nicotine bitartrate (Sigma

Pharmaceuticals) dissolved in physiological saline. On day 13, animals were assigned to nicotine groups using their body weights, to ensure that all groups had a similar starting average body weight within approximately 10 grams of each other. Rats that were more than two standard deviations from the mean weight of their respective sex (male, female) were assigned to the saline group. On day 14, all animals were anesthetized using a 5% isoflurane/oxygen mixture and were maintained in anesthesia throughout the surgical implantation of the minipumps. The minipump equations and implantation procedures were based on Grunberg (26). A small incision was made between the shoulder blades of the rat and the minipump was inserted into the skin fold made by the surgical cut. The incision was then closed using surgical wound clips and the animal was returned to its home cage for monitoring and recovery. Recovery from surgery was determined by the animal fully re-animating from the anesthesia. The animals were subsequently returned to their housing rooms and assessed by USU LAM veterinary personnel for potential complications from surgery. Completion and recovery from surgery marked the conclusion of Phase 1.

## **Phase 2: Post-implant**

Phase two lasted for 18 days following the surgical implant of osmotic minipumps and concluded on day 32 with sacrifice by decapitation and biological specimen (trunk blood and brain tissue) collection. Post-implant OFA and body weight measurements were conducted weekly in a manner identical to Phase 1. For animals in the stress condition, exposure to environmental and predatory stress was identical to Phase 1.

## **Housing**

All rats were individually housed in standard polycarbonate shoebox cages (42.5 x 20.5 x 20 cm) with hardwood chip bedding (Pine-Dri). Subjects were single housed to minimize the effect of social enrichment on biobehavioral outcomes which has been reported in previous investigations (18; 51). Cage bedding was changed twice a week by USU LAM Husbandry staff to ensure animal health and to minimize environmental stress from unsanitary housing conditions. Animals had *ad libitum* access to standard, bland, laboratory chow (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. Housing rooms were kept at 23°C with 40% relative humidity. Because rats are nocturnal animals, the housing rooms were kept on a 12 hr reverse light cycle (0600-1800 lights off) to synchronize the active part of the rat circadian cycle with the human work day (49). Male and female animals were housed in separate rooms in order to minimize potential confounds from rat estrus cycle. The study was conducted under an approved USUHS Institutional Animal Care and Use Committee (IACUC) protocol (MPS-10-509) and conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

## **Activity Chambers**

Open field activity (OFA) is a measurement of the animal's natural and unconditioned locomotor activity when placed into a darkened environment and left unperturbed for a set period of time (typically 60 minutes). OFA has been used in investigations of stress, affect, and nicotine using rat models (17; 18; 22; 27; 28; 31; 68). Data obtained from open field activity offer indices of overall health, anxiety-related and depression-related behaviors.

OFA was measured using Accuscan Superflex Sensor Version 2.2 infrared photocell system in sixteen Accuscan Instruments Standard Animal Cages (measuring 40 x 40 x 30 cm; Accuscan Instruments Incorporated, Columbus, OH) located in a separate room from the animals' housing room. The OFA room is designed and constructed with materials to minimize external acoustic disruptions that may create unwanted variance. The Standard Animal Cage is constructed of polycarbonate with a ventilated, removable Plexiglas lid that allows adequate airflow while preventing the animal from escaping during the trial. The animal's movement is tracked by three, paired 16-photocell Superflex Sensors which continuously transmit the location data to the Accuscan Superflex Node located on the upper-rear of the Standard Animal Cage. The Superflex Node transmits the OFA data to a central desktop computer through a universal serial bus (USB) connector. The data from the sixteen chambers are processed and aggregated by Accuscan Fusion Software (Version 3.4) and were converted into exportable HyperText Markup language (HTML) for further data interpretation and analysis (67; 68). The open field activity of each rat was measured for 60 minutes during the animal's active period (dark cycle) on days 2 [acclimation], 3 [baseline], 16 [time 1], 23 [time 2], 29 [time 3] etc. The OFA equipment begins recording data immediately after the rat was placed into their respective Standard Animal Cage. The experimenter then exited the test room and turned off the testing room's overhead red light leaving the animal in complete darkness during the trial. After the 60 minute testing trial concluded, the animals were retrieved from the Standard Animal Cage and returned to their home cages in the housing rooms. Boxes were cleaned and deodorized with Clidox (Pharmacal, Naugatuck, CT) solution between each subject.

## **Warrior Stress**

The Warrior Stress Paradigm (WSP) for rats was designed by the Grunberg Lab to model both anticipatory stress and the unpredictable environmental stressors that Warriors often experience when living in a combat environment (67). The paradigm uses a model of predator stress through the use of synthetic fox urine (Buck Stop, Stanton, MI), which has been successfully used in previous investigations into stress using animal subjects (5; 31; 67). The WSP also includes both non-painful, unpredictable environmental and sensory stressors to simulate the significant anticipatory stress that Warriors face while deployed. These environmental stressors also keep them animals from habituating to the fox urine. Stressing was conducted in laboratory space physically separate from the subjects' housing rooms to limit exposure to environmental stressors for animals in the unstressed conditions. For male and female rats in the stress condition, the animal was transferred from its home cage to the "stress cage" (29 x 18 x 12 cm) with a lid and no bedding in the bottom of the stress cage. The synthetic fox urine (10mL) was absorbed by a large cotton ball and individually placed in varying spots in the animal's stress cage. On the first stress day, animals were exposed to 20 minutes of fox urine in the "stress cage" and then returned to their home cages. On stress days 2-10 following 10 minutes of exposure to the fox urine, the urine-soaked cotton ball was removed and the animal remained in the "stress cage" and subsequently exposed to an additional 10 minutes of exposure to a single non-painful, environmental stressor. The environmental stressors used throughout the experiment included loud noises from a container of rattling coins, flashing the laboratory lights on and off in rapid succession, and individually shaking each stress cage to throw the animal off-balance. The entire 20

minute stress manipulation was conducted under bright florescent light in a laboratory separate from the housing room and the behavioral rooms. A detailed description of the WSP is included in Appendix A.

### **Independent Variables**

Experiment 1 consisted of four independent variables (IV): **sex** (male, female), **stress** (no stress, stress), **nicotine** dosage (0, 3, 6 mg nic/kg/day), and **time** (BL, T1, T2, T3).

**Sex.** Because biobehavioral differences in affect and stress response between male and females were a particular interest of this experiment, male and female rats were used.

**Stress.** American Warriors deployed to combat environments are often exposed to environmental stressors and face serious risk of death or serious injury. Deployed Warriors are at substantial risk for developing post-traumatic stress disorder (PTSD), substance abuse/dependency, and other clinical disorders including depression and anxiety (35; 36). Because it is often challenging to study the effects of combat stress among warriors, using an animal model of combat stress greatly enhances the ability to investigate the biobehavioral effects of combat stress and comorbid behavior (e.g., nicotine use). Additionally, an animal model allows for random assignment to condition and a true control group, which would not be possible in a human study.

**Nicotine.** In the first experiment, dosages of 0 (Saline control), 3, and 6 mg nic/kg/day were prepared based on the average weight of the subjects within each of the 12 treatment cells. Based on previous animal and human literature, these dosages model a non-smoker, a half pack/day smoker, and a pack/day smoker (26-28; 31; 64).

**Time.** Time was used as the within-subject independent variable to assess the effects of sex, nicotine, and stress at four time points within the experiment (BL, T1, T2, T3). Previous experiments performed in the Grunberg lab have observed significant biobehavioral effects in male and female subjects exposed to nicotine over time including differences in body weight, feeding behavior, and locomotion (17; 22; 28). Time as a within-subject IV facilitates the project's data analysis strategy by allowing observation of changes in the dependent variables over time. Additionally, time as a within-subject variable permits verification of the delivery of the nicotine to the animal by examining body weight.

### **Dependent Variables**

Dependent variables in this research project measured depression-related and anxiety-related behaviors using **Vertical Activity** and **Center Time**, respectively. Additionally, measurements of the animal's overall health were measured as **Horizontal Activity**. The following section describes the outcome variables measured and rationale for their use.

**Vertical Activity (VA).** Vertical activity measures an animal's rearing or natural escape behaviors. Previous studies have successfully used VA as a model of depression in rats (2; 32; 53; 67; 69) with rats that demonstrate less escape behavior showing more depression-related behavior. This model is largely based on Seligman's (54) learned helplessness paradigm where "non-depressed" animals demonstrate normal escape behavior. Vertical activity was measured as the number of times an animal broke a photoelectric beam generated on the upper half of the field apparatus.

**Center Time (CT).** Center time is a measurement of the animal's time spent in the center of the open field and is a long-established index of anxiety in rats (30-32; 41; 62; 67). The longer an animal spends in the center of the field correlates to less anxiety as anxious animals would be more likely to explore and stay on the periphery of the chamber (58). Center time was measured as a ratio of the time the animal spends in the center of the field over the total time the animal spends moving.

**Horizontal Activity (HA).** Horizontal activity provides a metric of the animal's gross motor movement and general health (18; 31; 32; 67). Horizontal activity is measured based on the number of times an animal broke a photoelectric beam generated on the lower half of the polycarbonate animal cage described above.

## CHAPTER 5: Results – Experiment 1

### Data Analytic Strategy

Using SPSS (IBM, 2013), repeated-measures analysis of covariance (rANCOVA) was conducted on each of the dependent variables (HA, VA, CT). In order to minimize Type-II error, treatment cell size and power analysis were based on previous empirical studies (5; 26; 28; 67; 68). Because some significant differences were observed among animals at baseline (BL), BL data were covaried throughout the data analysis and in the figures presented. This first rANCOVA was conducted to evaluate change over time. Data were split by sex and an additional rANCOVA was conducted to evaluate Hypothesis 3 (H3). Data were further split by stress before conducting additional rANCOVAs to evaluate Hypothesis 1 and 2 (H1, H2). In order to examine significant interactions revealed by the rANCOVAs, standard analyses of covariance (ANCOVAs) were conducted at each time point (T1, T2, T3) to examine each stress and sex subgroup independently (i.e., unstressed females, stressed females, unstressed males, stressed males). Tests were two tailed using  $\alpha=.05$ . Greenhouse-Geisser corrections are presented if a violation of sphericity was detected. Planned-comparisons of nicotine groups are presented as pairwise comparisons with mean differences (MD) between the two significant values. No post-hoc analyses were conducted. In interpreting the effect size ( $\eta^2$ ) of nicotine, Cohen's (11) convention of small (.01-.05), medium (.06-.13), and large (>.14) was used. Data presented in text includes only significant results. Means and Standard Errors (SEs) presented were computed from the model generated by SPSS (LSMeans). One female animal in the 3 mg nic /kg/ day, stress group was euthanized prior to time point 3 (T3). Missing data for this animal was replaced by an average of the

data for the treatment cell for HA, VA, and CT. All significant and non-significant data are presented in Appendix B.

### **Horizontal Activity (HA)**

#### Horizontal Activity Overall Repeated-measures Analysis of Covariance

The overall rANCOVA using all independent variables was conducted for HA. Figure 1 presents HA collapsed across sex to evaluate changes in HA over time. There was a main effect for time ( $F[2,166]=4.41$ ,  $p=.01$ ,  $\eta^2=.05$ ), such that T1 (12948.10,  $SEM=332.42$ ) was significantly greater than T2 (11809.00,  $SEM=376.23$ ) and T3 (11534.65,  $SEM=345.89$ ). The pairwise comparison of T1 and T2 was significant ( $MD=1139.10$ ,  $p<.01$ ) and the pairwise comparison of T1 and T3 was significant ( $MD=1413.46$ ,  $p<.01$ ). Figure 1a presents the HA data for T1, T2, and T3 collapsed across the other IVs (sex, stress, and drug condition). For Experiment 1, there also was a time x sex interaction ( $F[2,166]=4.50$ ,  $p=.01$ ,  $\eta^2=.05$ ), a time x nicotine interaction ( $F[4,166]=4.33$ ,  $p<.01$ ,  $\eta^2=.09$ ), and a time x sex x nicotine interaction ( $F[4,166]=2.70$ ,  $p=.03$ ,  $\eta^2=.06$ ). In order to interpret these results further, internal analyses were conducted.

#### Horizontal Activity Repeated-measures Analysis of Covariance Split by Sex

Data were split by sex and an additional rANCOVA was conducted. Figure 2 presents HA for female rats in Experiment 1. For female rats there was a main effect for time ( $F[2,82]=9.08$ ,  $p<.01$ ,  $\eta^2=.18$ ), such that T1 (15702.15,  $SEM=555.14$ ) was significantly greater than T2 (14186.69,  $SEM=657.93$ ) and T3 (13399.02,  $SEM=594.00$ ). Pairwise comparison of T1 and T2 was significant ( $MD=1515.46$ ,  $p<.01$ ) and pairwise comparison of T1 and T3 was significant ( $MD=2303.13$ ,  $p<.01$ ). Figure 2a shows HA

for female rats at each time point collapsed across stress and drug condition. For female rats there was a time x nicotine interaction ( $F[4,82]=3.92$ ,  $p<.01$ ,  $\eta^2=.16$ ), such that HA initially increased at T1 and then decreased over time for female rats in the nicotine treatment groups. Figure 2b shows the time x nicotine interaction for female rats collapsed across stress condition. There was no main effect of stress or stress interactions.

For male rats there was no main effect for time. Figure 3 shows the HA for male rats in Experiment 1. For male rats, there was a significant time x nicotine interaction ( $F[4,82]=2.83$ ,  $p=.03$ ,  $\eta^2=.12$ ), such that rats in the 3 mg/kg group demonstrated greater HA at first, but decreased over time. Pairwise comparisons of the three drug conditions were not significant. Figure 3a shows the time x nicotine interaction for males collapsed across stress condition.

#### Horizontal Activity Repeated-measures Analysis of Covariance, Split by Sex and Stress

Data were split by sex and stress and a final rANCOVA was conducted. For unstressed female rats (see Figure 2), there was a main effect of time ( $F[2,40]=7.01$ ,  $p<.01$ ,  $\eta^2=.26$ ) such that T1(16423.38, SEM=741.18) and T2(15049.58, SEM=953.40) were significantly greater than T3(13417.79, SEM=702.39). For unstressed female rats, comparison of T1 and T3 were significant (MD=3005.58,  $p<.01$ ); pairwise comparison of T2 and T3 were significant (MD=1631.74,  $p=.02$ ) across drug groups. For unstressed female rats, pairwise comparison of saline and 3 mg nic/kg/day were significant (MD=-3925.03) across time points. For stressed female rats, pairwise comparison of T1 and T2 were significant (MD=1657.12,  $p<.01$ ) across drug groups. For unstressed males (see Figure 3) there were no significant pairwise comparisons. For stressed males, there were no significant pairwise comparisons.

### Horizontal Activity Analysis of Covariance (T1, T2, T3)

For each time point (T1, T2, T3) a standard analysis of covariance (ANCOVA) was conducted on each subgroup using stress and sex as within-subjects variables. For unstressed females at T1 (see Figure 4), the ANCOVA revealed a main effect for nicotine ( $F[2,20]=5.54$ ,  $p=.01$ ,  $\eta^2=.36$ ), such that the saline group (12983.44,  $SEM=1298.47$ ) was significantly less than the 3 mg nic/kg/day group (18837.62,  $SEM=1283.82$ ) and the 6 mg nic/kg/day group (17449.07,  $SEM=1296.82$ ). For unstressed females at T1, pairwise comparison of saline and 3 mg nic/kg/day group was significant ( $MD=-5854.18$ ,  $p<.01$ ) and pairwise comparison of saline and 6 mg nic/kg/day group was significant ( $MD=-4465.64$ ,  $p=.03$ ). The effect of nicotine for unstressed females at T1 can be interpreted as large based on Cohen's (11) effect size convention. Figure 4 shows the main effect for nicotine at T1 for unstressed females.

For stressed females at T1, the ANCOVA did not reveal any significant findings. For unstressed males at T1, the ANCOVA did not reveal any significant findings. For stressed males at T1 (see Figure 5), the ANCOVA revealed a main effect for nicotine ( $F[2,20]=5.55$ ,  $p=.01$ ,  $\eta^2=.36$ ) such that the saline (9473.44,  $SEM=828.03$ ) and the 6 mg nic/kg/day (10468.70,  $SEM=828.17$ ) was significantly less than the 3 mg nic/kg/day (13233.35,  $SEM=827.04$ ). For stressed males at T1, pairwise comparison of saline and 3 mg nic/kg/day was significant ( $MD=-3759.91$ ,  $p<.01$ ) and pairwise comparison of 3 mg nic/kg/day and 6 mg nic/kg/day was significant ( $MD=2765.65$ ,  $p=.03$ ). The effect of nicotine for stressed males at T1 can be interpreted as a large effect size. Figure 5 shows the main effect for nicotine for stressed males at T1.

The ANCOVA did not reveal significant findings for any groups at T2 or T3.

### Summary for Horizontal Activity

For female rats, there was a significant time x nicotine interaction, such that for the nicotine groups, HA increased at first, and then decreased over time. For male rats, there was a significant time x nicotine interaction, such that only for the 3 mg/kg group, HA increased at first, then decreased over time. For female rats in both stress conditions, nicotine appears to have an initial activating effect, which decreases over time. This activating effect (HA) is more clearly seen in unstressed females than stressed females. For unstressed female rats, the 3 mg nic/kg/day group demonstrates more HA throughout Experiment 1 than saline controls. For stressed male rats, the activating effect of nicotine is only observed at T1, and only in the 3 mg nic/kg/day group.

### **Vertical Activity (VA)**

#### Vertical Activity Repeated-measures Analysis of Covariance, Overall

The overall rANCOVA using all independent variables was conducted for VA (see Figure 6). There was no significant main effect for time. Figure 6 presents VA for all animals at T1, T2, and T3 collapsed across sex.

For Experiment 1, there was a time x sex interaction ( $F[2,166]=3.814$ ,  $p=.02$ ,  $\eta^2=.04$ ); and a time x sex x stress interaction ( $F[2,166]=3.26$ ,  $p=.04$ ,  $\eta^2=.04$ ). Subsequent internal analyses were conducted.

#### Vertical Activity Repeated-measures Analysis of Covariance, Split by Sex

Data were split by sex and an additional rANCOVA was conducted. For female rats, there was no main effect of time (see Figure 7). Figure 7 presents VA for all female rats in Experiment 1.

Although there was no significant main effect for time, for female rats there was a significant time x stress interaction ( $F[2,82]=3.20$ ,  $p=.05$ ,  $\eta^2=.07$ ). Figure 8 presents the time x stress interaction for female rats in Experiment 1 collapsed across drug group.

For male rats, there was no significant main effect of time and no significant interactions revealed. Pairwise comparisons between T1 and T2 were significant ( $MD=-160.71$ ,  $p=.02$ ) and pairwise comparisons between T1 and T3 were significant ( $MD=-231.83$ ,  $p=.01$ ) collapsed across all stress and drug groups. Pairwise comparisons between unstressed and stressed male rats were significant ( $MD=-235.86$ ,  $p=.01$ ) collapsed across drug groups. Figure 9 presents the VA for males in Experiment 1.

#### Vertical Activity Repeated-measures of Covariance, Split by Sex and Stress

Data were split by sex and stress and a final rANCOVA was conducted. Within the unstressed female group, pairwise comparison reveals that T2 had greater VA than T3 ( $MD=252.58$ ,  $p<.05$ ) across all drug treatment groups. For unstressed female rats, pairwise comparison reveals that the 6 mg nic/kg/day group had more VA than saline controls throughout Experiment 1 than saline controls ( $MD=462.52$ ,  $p<.05$ ) across all time points. For stressed female rats there were no significant pairwise comparisons. For unstressed male rats, pairwise comparisons of T1 and T2 were significant ( $MD=-195.75$ ,  $p=.03$ ) and pairwise comparisons of T1 and T3 were significant ( $MD=334.83$ ,  $p<.01$ ) irrespective of drug treatment group. For stressed males there were no significant pairwise comparisons.

### Vertical Activity Analysis of Covariance (T1, T2, T3)

For each time point (T1, T2, T3) a standard analysis of covariance (ANCOVA) was conducted on each subgroup using stress and sex as within-subjects variables. The ANCOVA did not reveal any significant findings for any subgroup at T1, T2, or T3.

### Summary for Vertical Activity

Pairwise comparisons revealed that unstressed female rats demonstrated more VA at T3 than at T1 or T2, regardless of drug condition. Unstressed female rats administered 6 mg nic/kg/day demonstrated more VA at T1, T2, and T3 than saline controls. Unstressed male rats demonstrated increasing VA from T1 to T3 irrespective of drug group. Nicotine did not have an effect on VA in Experiment 1.

### **Center Time (CT)**

#### Center Time Repeated-measures Analysis of Covariance, Overall

The overall rANCOVA using all independent variables was conducted for CT. There was a main effect for time ( $F[2,166]=6.11$ ,  $p<.01$ ,  $\eta^2=.07$ ), such that T1 (10.70, SEM=.73) was significantly less than T2 (12.38, SEM=.59) and T3 (13.53, SEM=.73). For all animals, pairwise comparisons of T1 and T2 were significant ( $MD=-1.67$ ,  $p=.02$ ) and pairwise comparisons for T1 and T3 were significant ( $MD=-2.82$ ,  $p<.01$ ). Figure 10 presents center time data for all animals at T1, T2, and T3 collapsed across sex. No significant interactions were revealed. Subsequent internal analyses were conducted.

For male rats, there was a significant main effect of time ( $F[2,82]=3.93$ ,  $p=.02$ ,  $\eta^2=.09$ ) such that T1(8.36, SEM=.53) was significantly less than T2(10.77, SEM=.70) and T3(11.73, SEM=.87) (see Figure 12). For male rats, pairwise comparisons between T1 and T2 were significant ( $MD=-2.42$ ,  $p<.01$ ) and pairwise comparisons between T1

and T3 were significant ( $MD=-3.37$ ,  $p<.01$ ) collapsed across stress and drug groups.

Figure 12 presents the CT for male rats in Experiment 1. For male rats, there were no significant interactions revealed.

#### Center Time Repeated-measures Analysis of Covariance, Split by Sex and Stress

Data were then split by sex and stress and a final rANCOVA was conducted. There were no significant findings for unstressed female rats, stressed female rats, and unstressed male rats. For stressed male rats, there was a significant effect of time ( $F[2,40]=4.80$ ,  $p=.01$ ,  $\eta^2=.19$ ) such that T1(8.79,  $SEM=.75$ ) was significantly less than T2(11.09,  $SEM=.99$ ) and T3(11.49,  $SEM=1.06$ ). Figure 13 presents the CT for stressed male rats in Experiment 1.

For unstressed female rats, no significant comparisons were revealed. For stressed female rats, pairwise comparison of T1 and T3 were significant ( $MD=-3.94$ ,  $p=.02$ ) and pairwise comparison of T2 and T3 were significant ( $MD=-2.96$ ,  $p<.01$ ) irrespective of drug group. For unstressed males, pairwise comparison of T1 and T2 were significant ( $MD=-2.53$ ,  $p=.03$ ) and pairwise comparison of T1 and T3 were significant ( $MD=-4.04$ ,  $p<.01$ ) irrespective of drug group. For stressed male rats, pairwise comparison of T1 and T3 were significant ( $MD=-2.70$ ,  $p=.03$ ) irrespective of drug group.

#### Center Time Analysis of Covariance (T1, T2, T3)

For each time point (T1, T2, T3) a standard analysis of covariance (ANCOVA) was conducted on each subgroup using stress and sex as within-subjects variables. The ANCOVA did not reveal any significant findings for any subgroup at T1, T2, or T3

### Summary for Center Time

Stressed male and stressed female rats demonstrated increasing CT over time regardless of drug group. Nicotine did not have any significant effect on CT in Experiment 1.

## **CHAPTER 6: Evaluation of Hypotheses – Experiment 1**

Experiment 1 was conducted to examine sex and stress-related differences in anxiety-related and depression-related behavior in rats exposed to different dosages of nicotine. As stated in the introduction, nicotine has been observed to have both anti-depressant and anxiolytic properties in animals and humans. To interpret the findings from Experiment 1, the following discussion is divided between depression-related behavior and anxiety-related behavior. Hypotheses for Experiment 1 are re-stated for reference.

**H1:** Stressed rats will show more anxiety-related and depression-related behavior than unstressed rats.

**H2:** Nicotine will attenuate anxiety-related and depression-related behaviors based on the model and environment selected for the study.

**H3:** There will be a difference in the effects of nicotine between male and female rats.

**H4:** There will be a difference between anxiety-related and depression-related behavior between male and female rats- males will show less anxiety-related and depression-related behavior than female rats.

### **Depression-related Behavior**

Vertical Activity (VA) was the measurement of depression-related behavior for animals in Experiment 1, where VA is inversely related to depression-related behavior. All unstressed females demonstrated increasing levels of VA as the experiment progressed. A significant finding for unstressed females was observed in the 6 mg nic/kg/day group, which had higher VA throughout Experiment 1, however, this finding

was found in planned comparison with saline controls and not in the omnibus analyses. No clear, overall anti-depressant effects for nicotine was observed in Experiment 1.

### **Anxiety-related Behavior**

Center Time (CT) was the measurement of anxiety-related behavior for animals in Experiment 1, where increased CT indicates less anxiety-related behavior. The results indicated that both male and female stressed rats demonstrated more CT (i.e. less anxiety-related behavior) late in the experiment (T3), although without respect to drug group. Without differences between groups administered nicotine and saline controls, any anxiolytic effects of nicotine remain uncertain. Additionally, it is unclear if greater movement on the periphery of the OFA chamber was a result of the environmental and predatory stress (i.e., anxiety) or the activating (stimulant) effects of nicotine. Data from Horizontal Activity (HA) may help to differentiate anxiety-related behavior from overall active movement, however the interpretation of HA is also unclear. HA was higher in unstressed females than stressed females, although HA in both groups decreased over time. Although a significant finding for HA was revealed for stressed male rats at T1, the lack of any additional significant findings to compare with findings from CT limit interpretation of any anxiolytic effect of nicotine in Experiment 1. No clear anxiolytic effects of nicotine were observed in Experiment 1.

## CHAPTER 7: Discussion of Experiment 1

As stated in the introduction, the anxiolytic nature of nicotine is unclear (3) or highly variable depending on the environment and model of nicotine administration (46). Findings in Experiment 1 concerning anxiety-related behavior are similarly unclear and do not provide any meaningful interpretation about sex-related differences in nicotine use under stressful conditions. Nicotine may have genuine anxiolytic properties, or may be acting solely as a stimulant.

In contrast to anxiety, the anti-depressant effects of nicotine in humans are much clearer (38-40). Findings from Experiment 1 suggest that unstressed female rats are less depressed when administered nicotine at 6 mg nic/kg/day, a dose that models a single pack of cigarettes a day (26-29; 64). The anti-depressant effect of nicotine with unstressed female rats at 6 mg nic/kg/day was the most consistent significant preliminary finding, **but as this finding was found in planned comparison and not in the more conservative omnibus analyses, it should be only taken as a preliminary finding that warrants future study and replication.**

In summary, no clear sex, stress, or nicotine effects were observed in Experiment 1. Accordingly, **all of our hypotheses for Experiment 1 remain unconfirmed.**

## **CHAPTER 8: Overview and Hypotheses – Experiment 2**

### **Overview**

A second experiment was conducted to build on the most interesting findings of Experiment 1. That is, nicotine may decrease depression-related behavior in unstressed female rats. Therefore, to broaden understanding of any anti-depressant or anti-anxiety effects of nicotine on female rats, an additional treatment group of 9 mg nic/kg/day was added to model a 2 pack/day cigarette smoker (26-28; 64).

Experiment 2 examined effects of 4 dosages of nicotine (0, 3, 6, 9 mg nic/kg/day) in female rats. Experiment 2 included 6 animals each in the 0, 3, and 6 mg nic/kg/day groups and 14 animals in the 9 mg nic/kg/day group. Data from the unstressed female rats in Experiment 1 were combined with data from Experiment 2 for the 0, 3, and 6 mg nic/kg/day, so that each treatment group had 14 subjects for final data analysis. The total number of unstressed female rats in Experiment 2 was 32. Experiment 2 was divided into two phases: before and during nicotine/saline administration.

### **Hypotheses**

**H1:** Female rats that are administered nicotine will demonstrate less depression-related behavior than saline controls.

H1: Rationale: Based on findings from Experiment 1, it is expected that unstressed female rats will show less depression-related behavior when administered at least 6 mg nic/kg/day.

## **CHAPTER 9: Methods – Experiment 2**

### **Timeline**

The second experiment followed an identical timeline to Experiment 1, only without any stressing of the animals. After one day of acclimatization and gentling, rats were placed into the Open Field Apparatus (OFA) chambers for acclimation, with baseline (BL) OFA measured on day 3 (see page 8 for a detailed description). Additional OFA trials were measured on day 16 (Time 1 [T1]), day 23 (Time 2 [T2]), and day 29 (Time 3[T3]). **Appendix A** includes a timeline of Experiment 2.

### **Subjects**

Animals used for Experiment 2 were 32 female Sprague Dawley (SD) rats from Charles River Laboratories (Wilmington, Massachusetts). See page 7 for a detailed discussion and rationale for the choice of subjects.

### **Phase 1: Pre-implant**

Pre-implant phase followed a methodology identical to Experiment 1, only without exposure to environmental or predatory stress. Body weight was measured weekly using an electronic laboratory balance. Similar to Experiment 1, on day 13, animals were assigned to nicotine group based off body weight, so that all groups had similar starting body weight averages. On day 14, animals were anesthetized with a 5% isoflurane/oxygen mixture and were maintained in anesthesia throughout the surgical implantation of the minipumps. See pages 8-9 for a detailed description of the surgical procedure. Recovery from the anesthesia marked the conclusion of Phase 1.

## **Phase 2: Post-implant**

Post-implant phase followed an identical methodology to Phase 1. Phase two lasted for 18 days following the surgical implant of osmotic minipumps and concluded on day 32 with sacrifice by decapitation and biological specimen (trunk blood and brain tissue) collection.

## **Housing**

All animals were single-housed in conditions identical to Experiment 1. See page 9 for a detailed description of housing and husbandry.

## **Activity Chambers**

Experiment 2 used Open Field Activity (OFA) and the same equipment utilized in Experiment 1. See pages 10-11 for a detailed description of OFA and the activity chamber equipment.

## **Independent Variables**

Independent variables for Experiment 2 consisted of two variables: **nicotine** dose (0, 3, 6, 9 mg nic/kg/day), and **time** (BL, T1, T2, T3). See pages 12-13 for a detailed explanation of the independent variables.

## **Dependent Variables**

Dependent variables for Experiment 2 consisted of three variables: Horizontal Activity (HA), Vertical Activity (VA), and Center Time (CT). See pages 14-15 for a detailed explanation of the dependent variables.

## **CHAPTER 10: Results – Experiment 2**

### **Data Analytic Strategy**

Findings from Experiment 1 informed the methods and data analyses for Experiment 2 - namely the inclusion of only unstressed female rats as subjects. Data from unstressed female rats in Experiment 1 were combined with data obtained from Experiment 2 so that each treatment group had 14 subjects in the final analysis. Because some significant differences existed among animals at baseline for Experiment 1, the strategy of using an overall repeated-measures analysis of covariance (rANCOVA) was preserved for Experiment 2. A rANCOVA was conducted for each variable to assess main effects of time, nicotine, and any interaction effect. Any violation of sphericity (as indicated by a significant value for Mauchly's Sphericity Test) was corrected by presenting the Greenhouse-Geiser values. Cohen's (11) convention for interpreting partial eta-squared ( $\eta^2$ ) was used for Experiment 2. Planned analyses of nicotine groups are presented as pair wise comparisons. No post-hoc comparisons were conducted. Subsequent internal analyses were conducted at each time point using a one-way ANCOVA.

Planned comparisons of nicotine groups are presented as pairwise comparisons with mean differences (MD) between the two significant values. Data presented in text includes only significant results. All significant and non-significant data are presented in Appendix B.

## **Horizontal Activity (HA)**

### Horizontal Activity Repeated-measures Analysis of Covariance, All Unstressed Females

The overall rANCOVA using all independent variables was conducted for HA. Mauchly's test indicated that the assumption of sphericity had been violated,  $\chi^2(2)=8.12$ ,  $p=.02$ . Therefore, degrees of freedom were corrected using Greenhouse-Geiser estimates of sphericity ( $\epsilon=.87$ ). There was no main effect for time. The overall rANCOVA revealed a significant interaction of time x nicotine,  $F(1.74, 88.70)=2.48$ ,  $p=.04$ ,  $\eta^2=.13$ . There were no significant pair-wise comparisons. Figure 14 presents the HA for all unstressed female subjects in Experiment 1 and Experiment 2. Subsequent internal analyses at each time point were conducted.

### Horizontal Activity Analysis of Covariance: T1

The ANCOVA revealed a significant effect of nicotine at T1,  $F(3,51)=5.55$ ,  $p<.01$ ,  $\eta^2=.25$ , such that the saline (11636.62, SEM=1167.05) and the 9 mg nic/kg/day group (11172.76, SEM=1175.44) were significantly less than the 3 mg nic/kg/day (16892.98, SEM=1172.41) and the 6 mg nic/kg/day (15140.86, SEM=1166.23). The effect of nicotine at T1 can be interpreted as a large effect size.

### Horizontal Activity Analysis of Covariance: T2

The ANCOVA revealed a significant effect of nicotine at T2,  $F(3,51)=4.24$ ,  $p=.01$ ,  $\eta^2=.20$ , such that the saline (11646.55, SEM=1081.85) was significantly less than the 3 mg nic/kg/day (16679.71, SEM=1086.82) and the 3 mg nic/kg/day (16679.71, SEM=1086.82) was significantly greater than the 9 mg nic/kg/day (12308.31, SEM=1089.63). The effect of nicotine at T2 can be interpreted as a large effect size.

### Horizontal Activity Analysis of Covariance: T3

The ANCOVA did not reveal a significant finding for T3.

### Summary for Horizontal Activity

Nicotine had the greatest activating effect for rats at T1, but only in the 3 and 6 mg nic/kg/day groups rendering an inverted-U dose response curve. Rats in the 9 mg nic/kg/day did not differ significantly from saline controls. Large effect sizes for nicotine were revealed at T1 and T2.

### **Vertical Activity (VA)**

#### Vertical Activity Repeated-measures Analysis of Covariance, All Unstressed Females

The overall rANCOVA using all independent variables was conducted for VA. There was no main effect for time or any significant interactions revealed. Pairwise comparisons of T3 and T1 were significant ( $MD=187.57$ ,  $p<.05$ ). Figure 15 presents the VA for all unstressed female rats in Experiment 1 and 2. Subsequent internal analyses at each time point were conducted.

#### Vertical Activity Analysis of Covariance: T1

The ANCOVA revealed a significant effect of nicotine at T1,  $F(3,51)=4.83$ ,  $p=.01$ ,  $\eta^2=.22$ , such that the saline (1374.32,  $SEM=169.40$ ) and 9 mg nic/kg/day (1162.56,  $SEM=167.59$ ) groups were significantly less than the 3 mg nic/kg/day group (1972.91,  $SEM=167.45$ ). The ANCOVA also revealed that the 6 mg nic/kg/day group (1771.33,  $SEM=168.38$ ) was significantly greater than the 9 mg nic/kg/day group (1162.56,  $SEM=167.59$ ). The effect of nicotine at T1 can be interpreted as a large effect size.

### Vertical Activity Analysis of Covariance: T2 and T3.

The ANCOVA did not reveal any significant finding for T2 or T3.

### Summary for Vertical Activity

There was no main effect of nicotine on VA for unstressed female rats in Experiment 1 or 2.

### **Center Time (CT)**

### Center Time Repeated-measures Analysis of Covariance, All Unstressed Females

The overall rANCOVA using all independent variables was conducted for CT. There was a significant main effect for time,  $F(2, 102)=5.00$ ,  $p=.01$ ,  $\eta^2=.09$ , such that T1(11.19, SEM=1.20) and T2(12.48, SEM=.95) were significantly less than T3(14.47, SEM=1.08). Pairwise comparisons of T1 and T3 were significant (MD=3.28,  $p<.01$ ). Figure 16 presents CT for all unstressed female rats in Experiment 1 and Experiment 2. No significant interactions were revealed. Subsequent internal analyses at each time point were conducted.

### Center Time Analysis of Covariance: T1, T2, and T3.

The standard ANCOVA did not reveal any significant finding for T1, T2 or T3.

### Summary for Center Time

There was no significant effect of nicotine on CT for unstressed female rats in either experiment 1 or 2.

## **CHAPTER 11: Evaluation of Hypotheses – Experiment 2**

Experiment 2 was conducted to build upon findings from Experiment 1 as they relate to unstressed female rats at varying dosages of nicotine. Additionally, Experiment 2 included a higher dose (9 mg nic/kg/day) to model high frequency human smokers and to enable plotting of a dose response curve. The hypothesis for Experiment 2 is restated for reference.

**H1A:** Female rats that are administered nicotine will demonstrate less depression-related behavior than saline controls.

### **Depression-Related Behavior**

Analysis of data from Experiment 1 and 2 did not yield any significant anti-depressant effects of nicotine. Some very preliminary data from planned comparisons may indicate that nicotine has time-limited anti-depressant effects in unstressed female rats in the 3 and 6 mg/kg/day dosages, but the lack of a main effect of nicotine renders hypothesis H1A unconfirmed.

### **Anxiety-Related Behavior**

The data did not indicate anxiolytic effects for unstressed female rats in either experiment 1 or 2.

## **CHAPTER 12: Discussion of Experiment 2**

Unstressed female rats that were administered low and moderate doses (3 and 6 mg nic/kg/day) in Experiment 1 and 2 demonstrated less depression-related behavior than saline controls or rats in the high (9 mg nic/kg/day) dose at T1 and T2 in the study. However, since these findings were found in planned comparisons and not in the more statistically robust omnibus analysis, these findings should be interpreted very carefully and warrant more study and replication. Since no main effects for nicotine was observed in Experiment 2, the hypothesis for this experiment remains unconfirmed.

## CHAPTER 13: General Discussion

The preceding two experiments utilized an animal model to examine nicotine's effects on anxiety-related and depression-related behavior. In Experiment 1, the primary focus was to examine sex and stress-related differences in anxiety-related and depression-related behavior. No significant anxiolytic or anti-depressant effects for nicotine were observed. Some planned comparisons indicated that unstressed female rats the moderate (6 mg nic/kg/day) dosages of nicotine had less depression related behavior than saline controls, but the lack of a significant main effect of nicotine limits interpretation of this finding.

Anxiety-related behavior, although more challenging to interpret than depression-related behavior, did show some significant effects for unstressed female rats in the low and moderate dosages, although these effects were at different time points in the two experiments and were revealed by statistically less-conservative planned comparisons. Unstressed female rats administered the lowest dosage of nicotine (3mg nic/kg/day) had more CT at T1 than did other treatment groups. By T3, the most CT was observed in unstressed female rats in the moderate dosage group (6 mg nic/kg/day). In examining the planned comparisons, there were no significant difference between unstressed female rats in the high dosage (9mg nic/kg/day) and the saline control group. Although these findings should be treated with caution, future investigations may reveal that there is a therapeutic window of anxiolytic effects of nicotine in unstressed female rats.

It is important to note in both measures of anxiety and depression that the most significant and interesting findings of nicotine was limited to female rats not exposed to environmental and predatory stress. The measure of depression-related and anxiety-

related behavior among these rats is limited to measuring the naturally-occurring behavior of rats when placed in an open field without additional environmental stressors.

Despite the experimental absence of predatory and environmental stress among unstressed female rats, there still may be some naturally-occurring anxiety-related behavior. Previous investigations have revealed that rats prefer to place themselves next to the edge or wall of the open field apparatus (OFA), a phenomenon (thigmotaxis) that Treit and Fundytus (58) suggest is an innate fear response, even in the absence of additional stressors. Increased time spent in the center is evidence of less anxiety-related behavior which was observed among unstressed female rats administered low and moderate dosages of nicotine. Similarly, the measure of depression-related behavior in Experiment 1 and 2 is based on Seligman and Beagley (54) “Learned Helplessness Paradigm,” which asserts that decreased rearing or escape behavior (vertical activity) of a rat placed in the OFA is a behavioral index of depression-related behavior. As with thigmotaxis, vertical activity occurs even without external interventions. Accordingly, findings from Experiment 1 and 2 may generalize to human female warriors in non-stressful conditions (i.e., garrison or state-side duty).

### **Use of Animal Model**

Nicotine studies using human subjects are often limited by self-report, recall-bias, and ethical considerations that prevent administration of addictive nicotine to previously naïve subjects (26-28; 31). Additionally, studies that examine male and female Warriors that are exposed to combat stressors are especially difficult to conduct in real-time when considering the security of the Warrior and investigator in a combat environment (32; 67; 68). The use of the animal model and stress paradigm as in this investigation allowed a

novel investigation into sex-related differences of anxiety-related and depression-related behaviors using varying levels of nicotine with semi-random subject assignment.

Although no significant stress, sex, or nicotine effects were revealed, some preliminary findings from planned comparisons may inform future investigations into nicotine use among Warriors using animal models. As stated above, these findings should be treated cautiously and require additional study and replication before any interpretation about human Warrior nicotine use in stressful conditions can be generalized.

### **Limitations**

The main limitation of the current study is that each measure of anxiety- and depression-related behavior is linked to animal movement and dosage of nicotine. In terms of Vertical Activity (VA), it is possible that the nicotine may be acting as an activating stimulant as well as a “traditional” anti-depressant. Another well-established measurement of depression-related behavior in rats is the Forced Swim Test (FST), although this too is a measure of movement within the same field (48). Because Experiment 1, which contained a stress condition, did not establish a clear relationship between center time (CT) and nicotine, no conclusion of the anxiolytic effect of nicotine in male and female rats can be made.

A second limitation of the study is the singular use of red fox urine within the Warrior Stress Paradigm (WSP). Although previous investigations have reported red fox urine to be a suitable model for predatory stress (10) other investigations have reported varied responses to different predator odors which may produce a greater stress effect than observed in the current study (1).

A third limitation of this study involved the use of combining the data from unstressed female rats in the first experiment to unstressed female rats in the second experiment. Although this combination of data was done to provide sufficient power used in previous investigations (5; 26; 28; 67; 68), there is the potential for inflation of non-significant results, although no significant sex, stress, or nicotine effects were observed in this study. Although subjects, housing, and experimental conditions between the two experiments were kept as similar as possible, this limitation should be addressed in future investigations.

A fourth limitation is the use of planned comparisons within the data analytic strategy, and not more conservative post-hoc corrections. Numerous planned comparisons may increase family wise error rate increasing the probability of Type-I error (37). The most interesting findings among unstressed female rats were observed among these comparisons, and should be treated as very modest that require further study and replication.

Finally, assignment to nicotine condition in Experiment 1 and Experiment 2 was not wholly random but rather semi-random based on the animal's weight. Although the animals are largely genetically identical, the lack of a true random assignment must be acknowledged as a limitation.

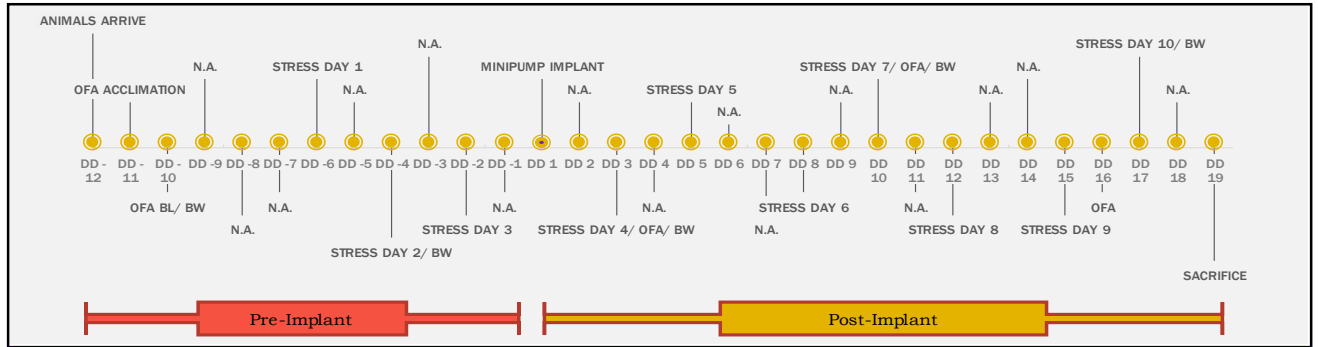
## **CHAPTER 14: Summary**

Tobacco use among Warriors in the United States continues to be a major concern to the Department of Defense and poses a serious threat to combat readiness. High levels of tobacco use continue to be measured among Millennial-generation Warriors, despite widespread knowledge of the health risks associated with tobacco use and the addictive properties of nicotine use (9). Such a counter-intuitive observation may be explained by examining the “beneficial” effects of nicotine consumption, especially when consumed in stressful environments. Additionally, future studies of sex-related differences in nicotine use should consider sex differences and social affiliation in pre-stress (or pre-deployment) smoking initiation, as males and females may be maintaining nicotine use under different learning and maintenance paradigms. The present study combined an animal model of sustained nicotine administration with a paradigm of Warrior stress to examine sex-related differences in anxiety-related and depression-related behavior.

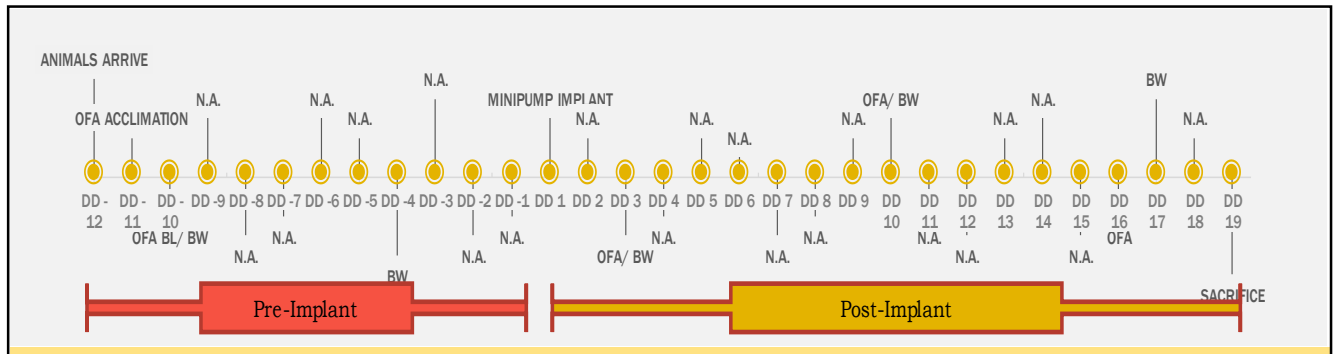
## **CHAPTER 15: Conclusions**

No clear sex or stress-effects were revealed in the project. Similarly, no clear anxiolytic or anti-depressant effects of nicotine were revealed in Experiment 1 or 2. Our hypotheses for this study remain unconfirmed.

## APPENDIX A- FIGURES



**Figure 1. Timeline of Experiment 1**



**Figure 2. Timeline of Experiment 2.**

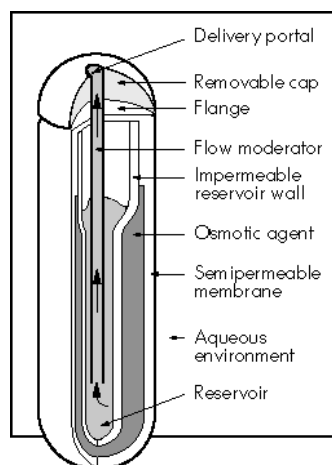
*Note.* “DD”-Drug Day, “N.A.”- No Activity, “BW”- Body Weight, “BL”-Baseline.

<b><u>Predatory Stressor</u></b>	<b><u>Procedure</u></b>
Fox Urine	Ensure each cotton ball has fully absorbed 15mL of synthetic fox urine before placing the urine-soaked ball into the stress cages for 10 minutes. At the end of 10 minutes, remove from the container and begin additional environmental stressors.
<b><u>Environmental Stressor</u></b>	<b><u>Procedure</u></b>
Main lights flash	Flash overhead lights six times using light switch randomly at 4 times within 10 minutes
Whistle	Blow whistle for 3-4 seconds randomly at 4 times within 10 minutes
Cage shaking	Leave cage on counter and shake front to back 5x vigorously randomly at 4 times within 10 minutes
Coins in metal container	Shake 5 times randomly 4 times within 10 minutes . The coin container should be held by the side to ensure proper sound.

**Figure 3. Warrior Stress Paradigm (WSP) Description and Procedure.**

STRESS DAY 1	PREDATOR STRESS 20 MINS
STRESS DAY 2	PREDATOR STRESS (REMOVE AFTER 10 MIN) THEN <b>WHISTLE</b> @ 12, 15, AND 19 MINS
STRESS DAY 3	PREDATOR STRESS (REMOVE AFTER 10 MIN) THEN <b>COIN SHAKE</b> @ 11, 14, AND 17 MINS
STRESS DAY 4	PREDATOR STRESS (REMOVE AFTER 10 MIN) THEN <b>FLASHING LIGHTS</b> @ 13, 16, 18, & 19 MINS
STRESS DAY 5	PREDATOR STRESS (REMOVE AFTER 10 MIN) THEN <b>CAGE SHAKE</b> @ 12, 15, AND 18 MINS
STRESS DAY 6	PREDATOR STRESS (REMOVE AFTER 10 MIN) THEN <b>FLASHING LIGHTS</b> @ 12, 16, & 19 MINS
STRESS DAY 7	PREDATOR STRESS (REMOVE AFTER 10 MIN) THEN <b>WHISTLE</b> @ 11, 13, 16, AND 18 MINS
STRESS DAY 8	PREDATOR STRESS (REMOVE AFTER 10 MIN) THEN <b>COIN SHAKE</b> @ 11, 14, AND 17 MINS
STRESS DAY 9	PREDATOR STRESS (REMOVE AFTER 10 MIN) THEN <b>FLASHING LIGHTS</b> @ 13, 16, 18, & 19 MINS
STRESS DAY 10	PREDATOR STRESS (REMOVE AFTER 10 MIN) THEN <b>WHISTLE</b> @ 11, 13, 16, AND 18 MINS

**Figure 4. Warrior Stress Paradigm Schedule.**



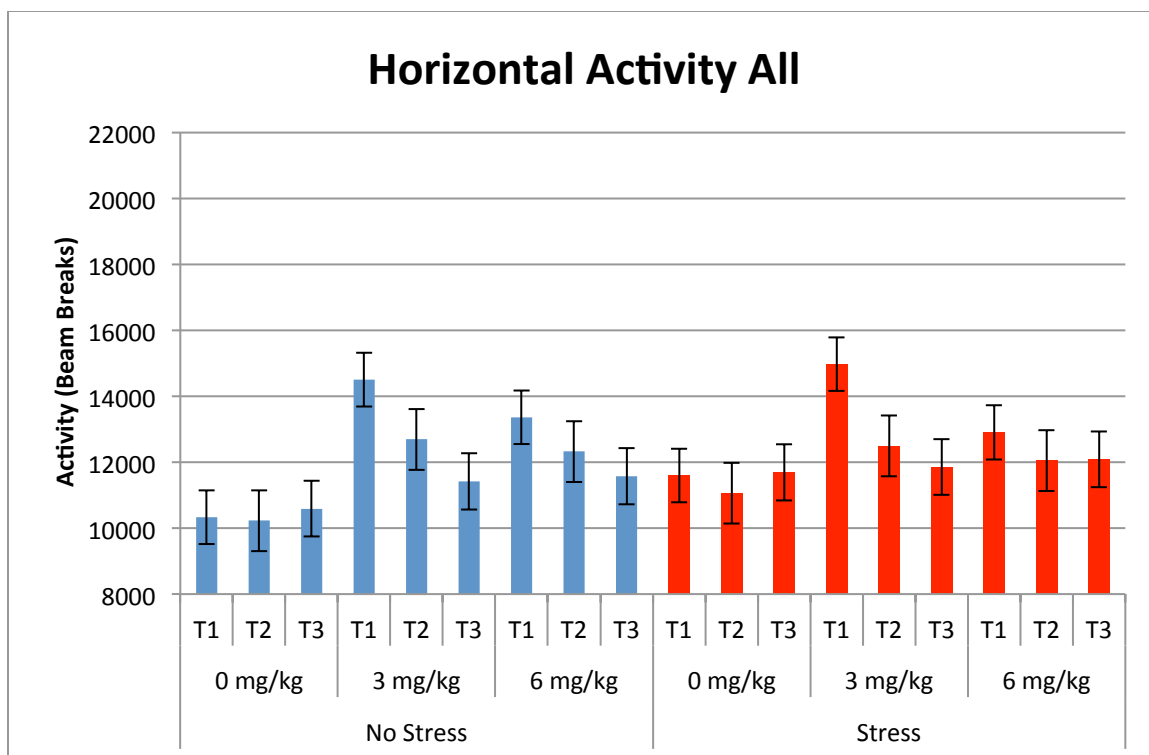
**Figure 5. Cross-section illustration of the ALZET Model 2002 Minipump**

Image by ALZET (2012)

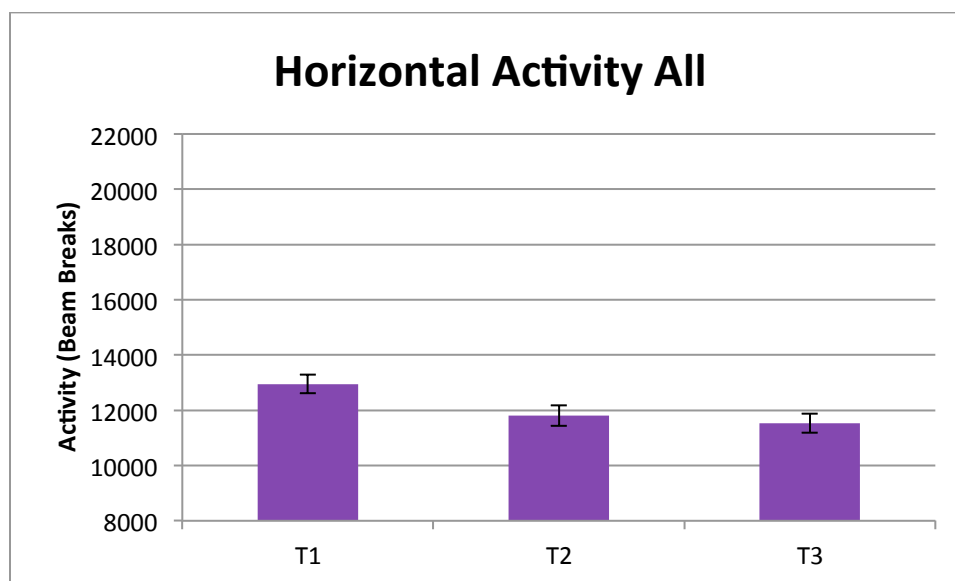


**Figure 6. Subcutaneous (SC) location of Minipump Implant in the Rat.**

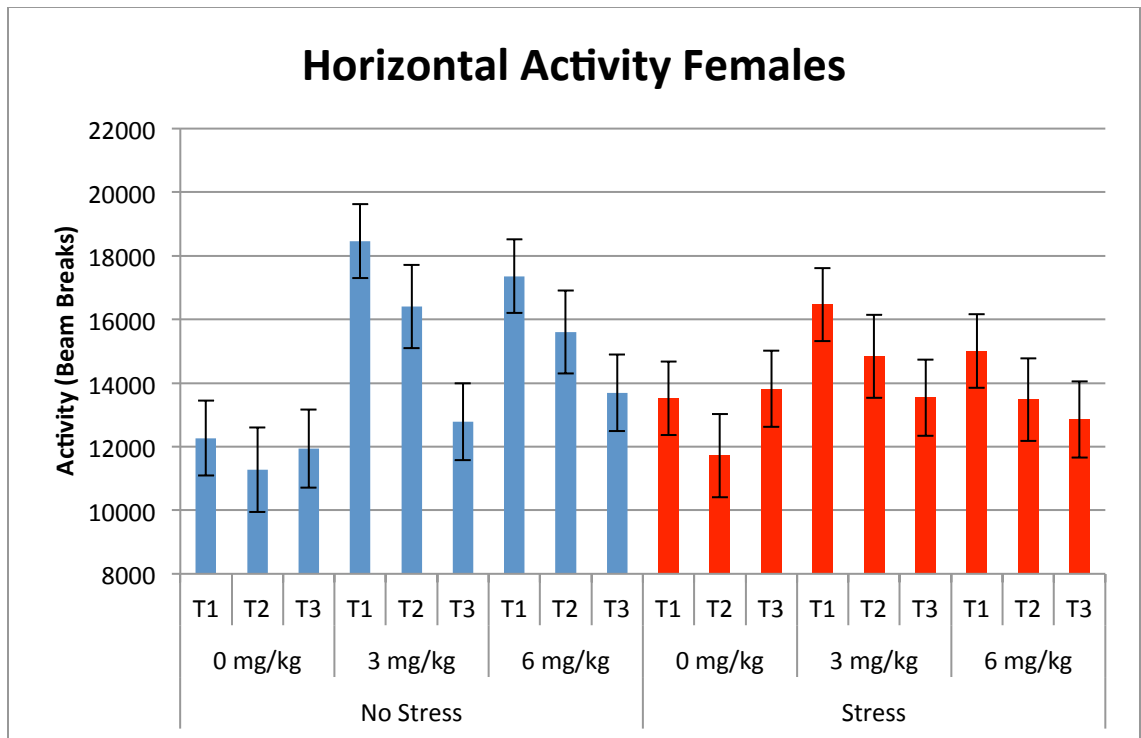
Image by ALZET (2012)



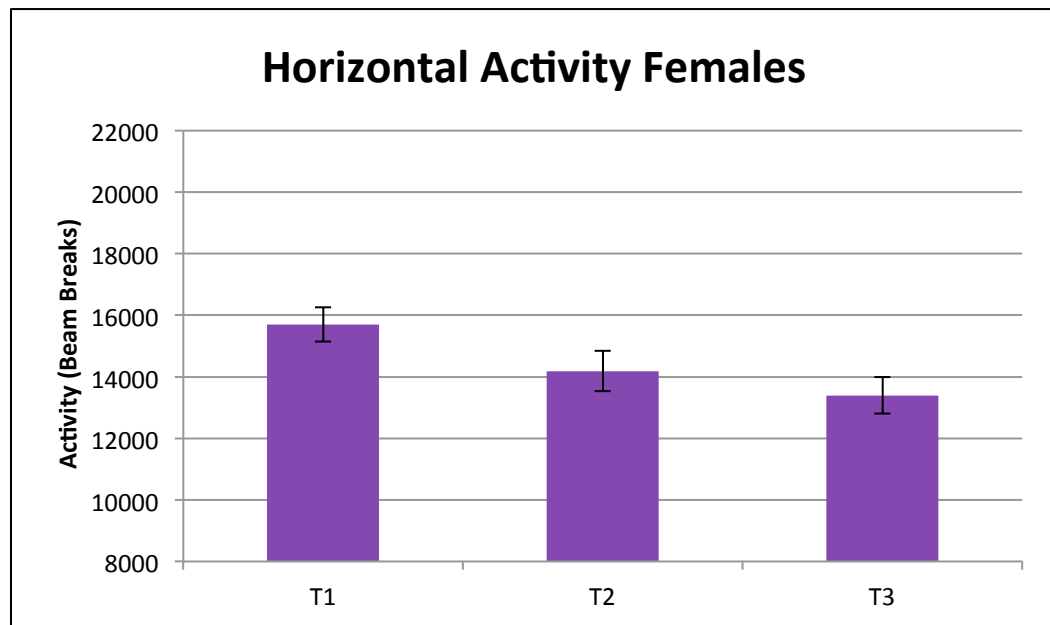
**Figure 7. Horizontal Activity, All- Experiment 1.**



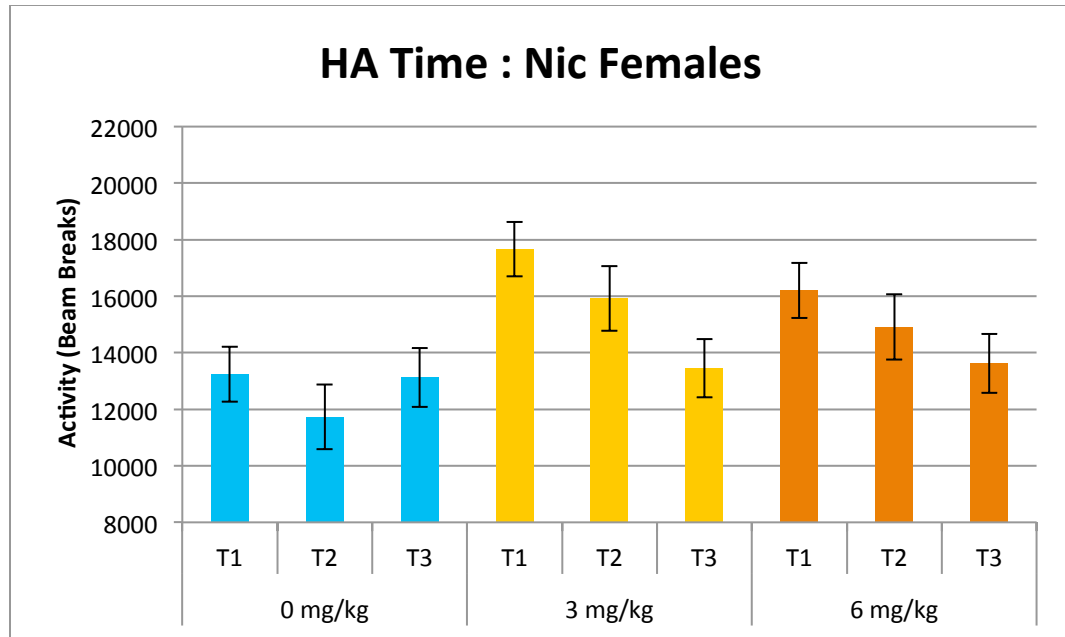
**Figure 8. Horizontal Activity, All, Collapsed Cx Sex and Stress- Experiment 1.**



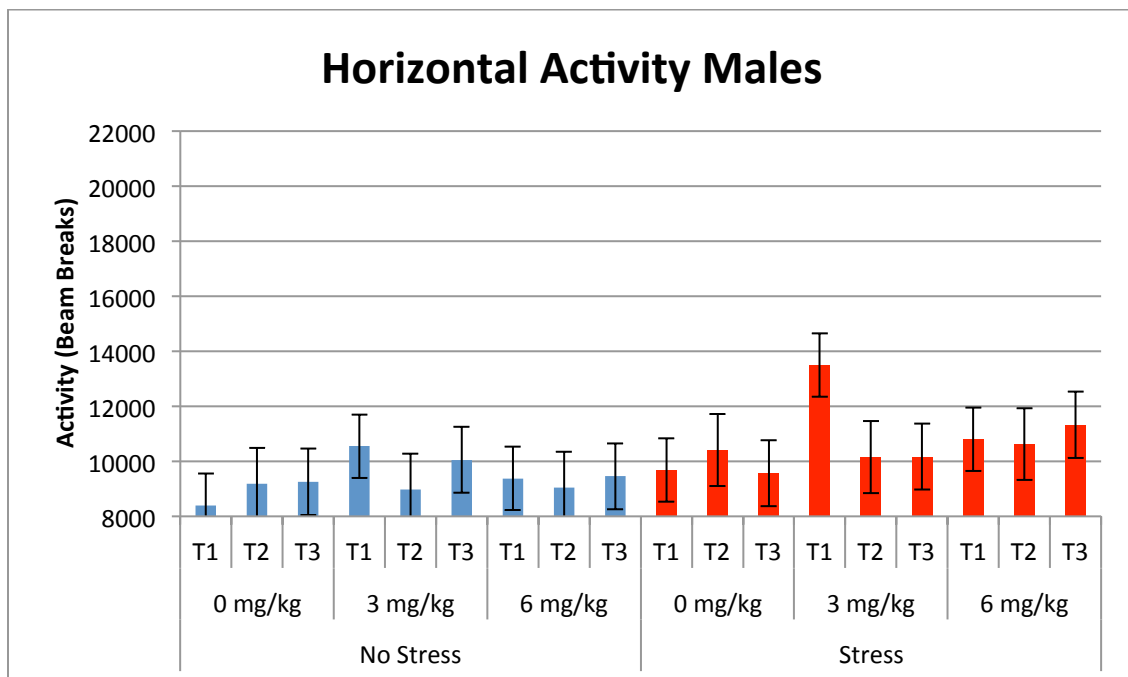
**Figure 9. Horizontal Activity, Females – Experiment 1.**



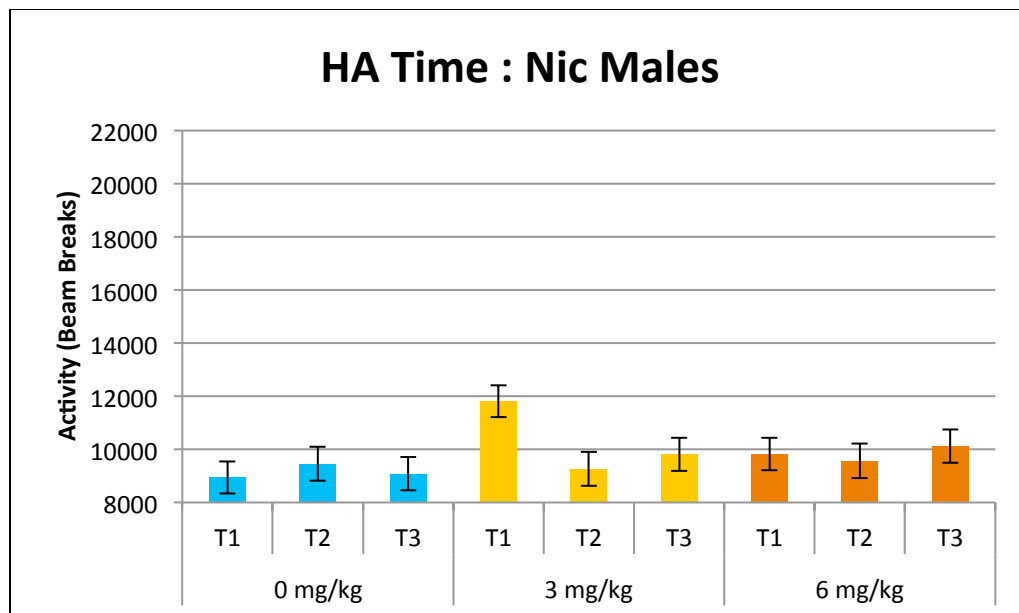
**Figure 10. Horizontal Activity, Females, Cx Sex and Stress – Experiment 1.**



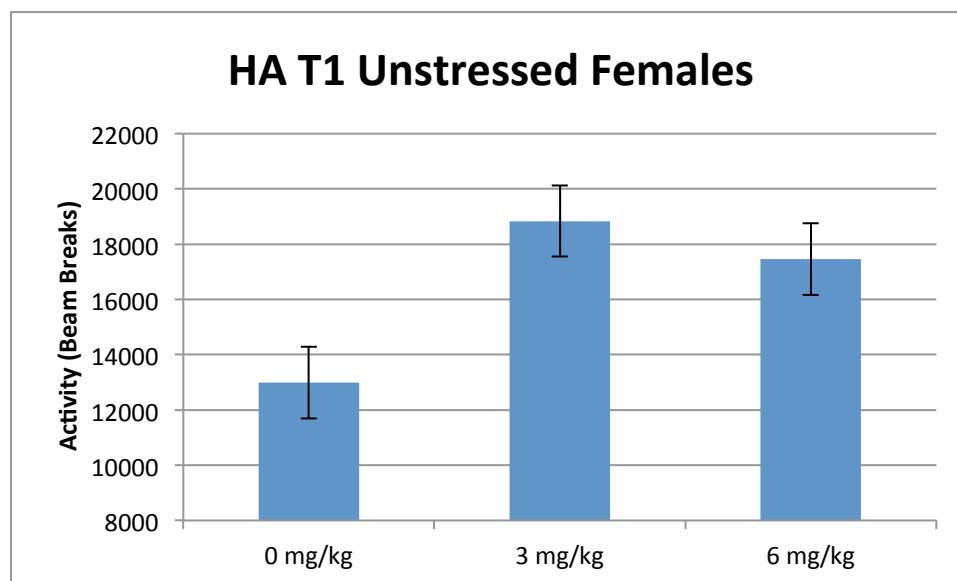
**Figure 11. Horizontal Activity, Females, Time : Nicotine – Experiment 1.**



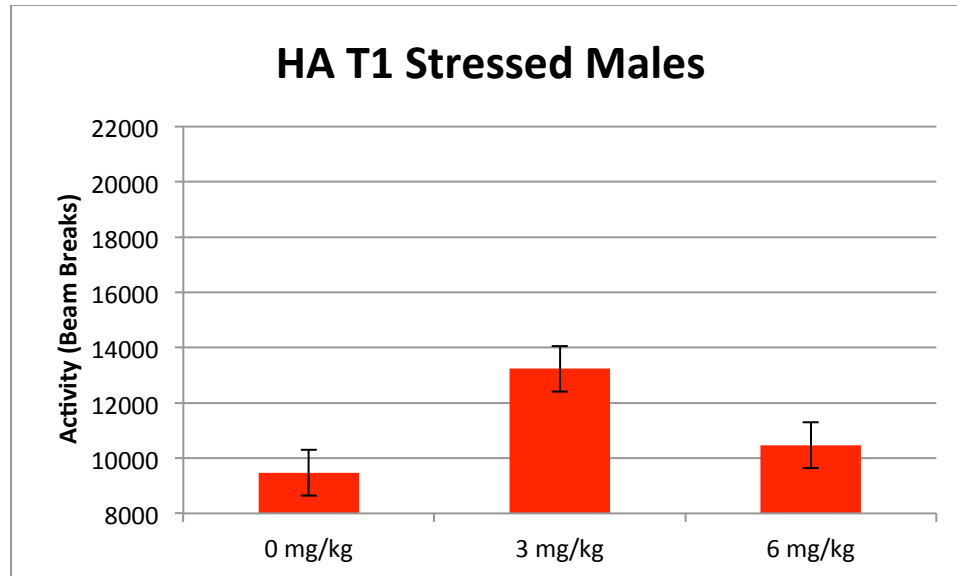
**Figure 12. Horizontal activity, Males – Experiment 1.**



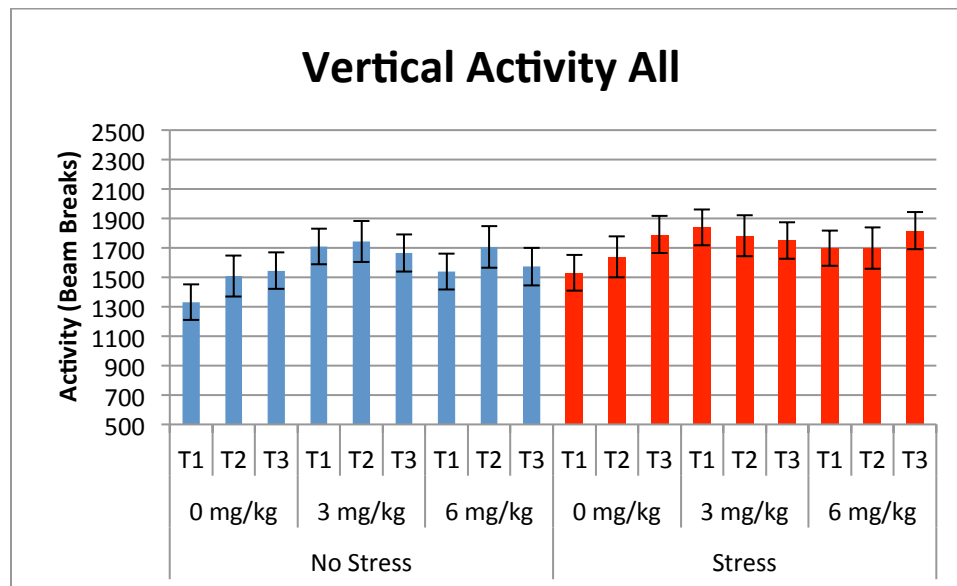
**Figure 13. Horizontal Activity, Males, Time : Nicotine – Experiment 1.**



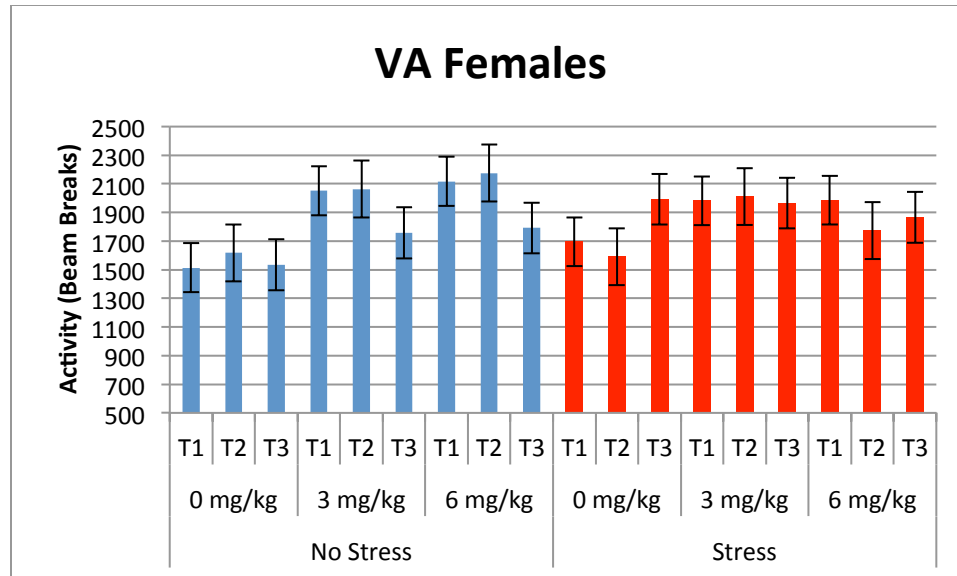
**Figure 14. Horizontal Activity, Unstressed Females, T1 – Experiment 1.**



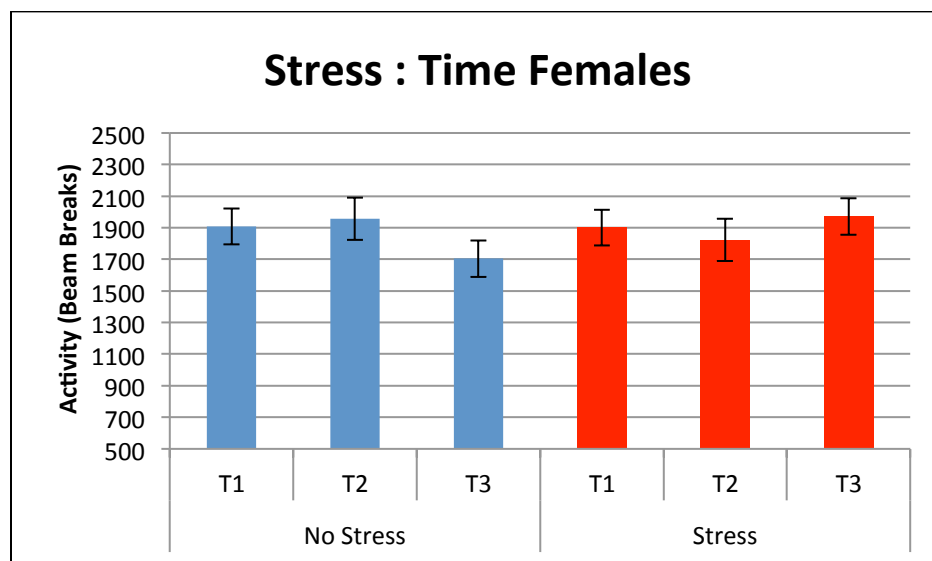
**Figure 15. Horizontal Activity, Stressed Males – Experiment 1.**



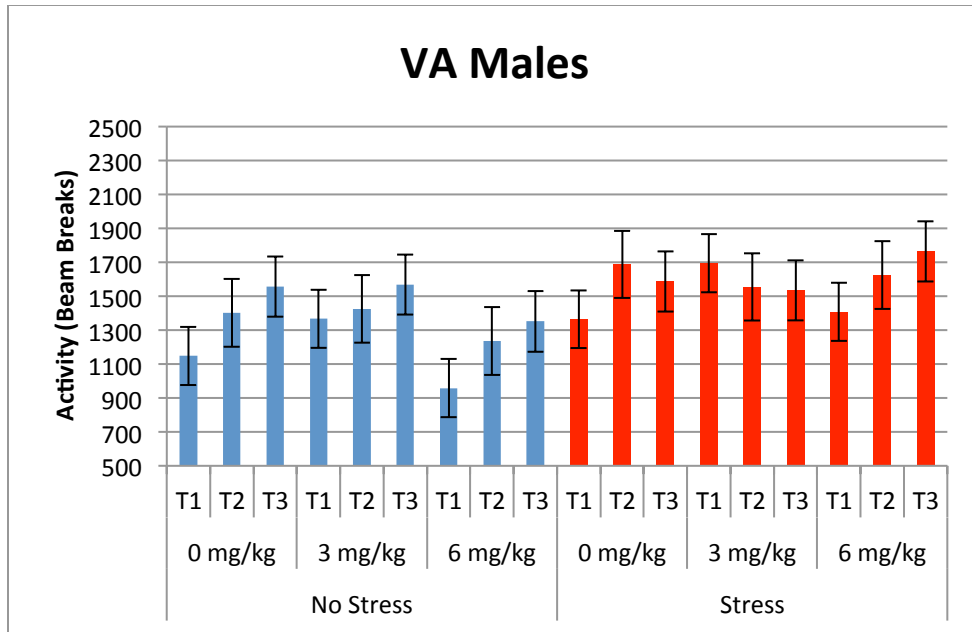
**Figure 16. Vertical Activity, All – Experiment 1.**



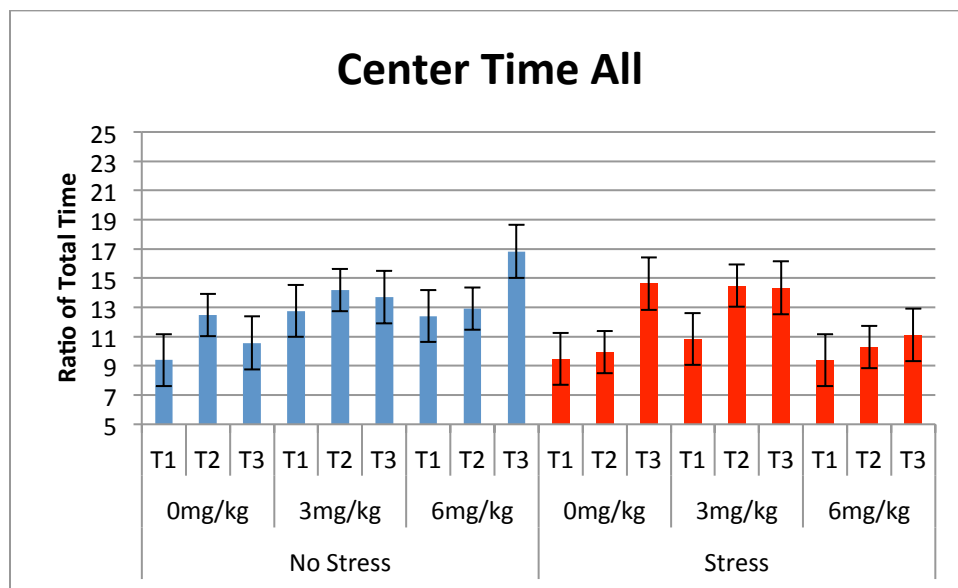
**Figure 17. Vertical Activity, Females – Experiment 1.**



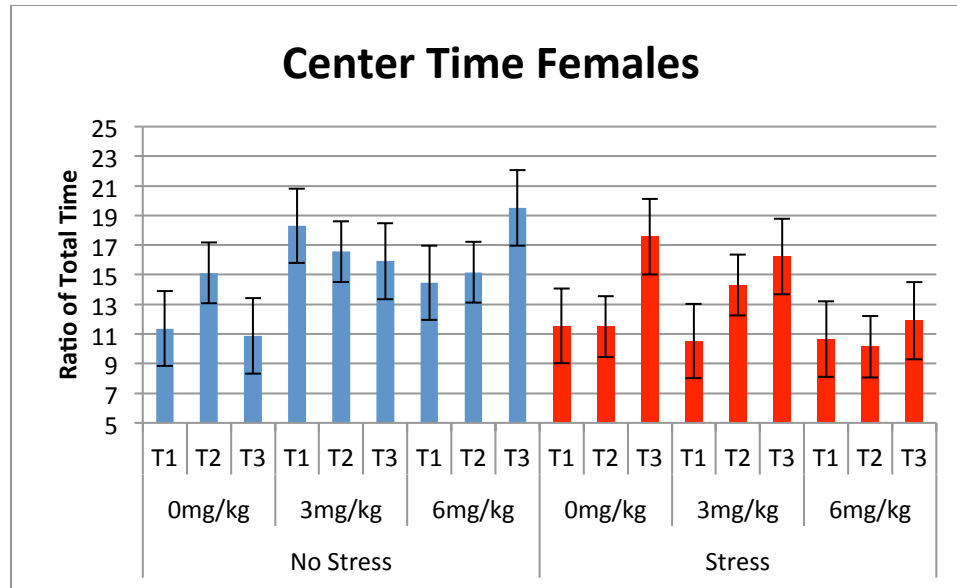
**Figure 18. Vertical Activity, Females, Stress : Time – Experiment 1.**



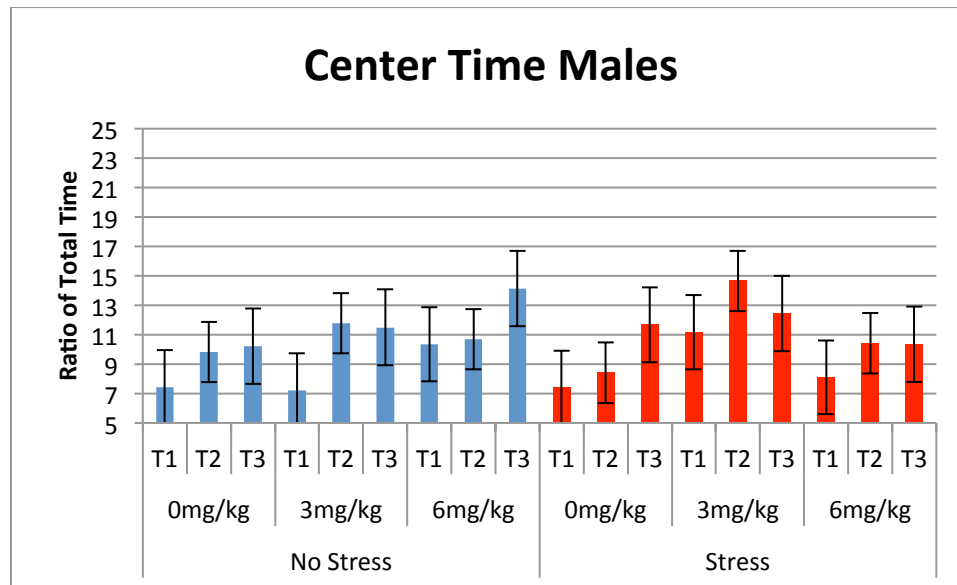
**Figure 19. Vertical Activity, Males – Experiment 1**



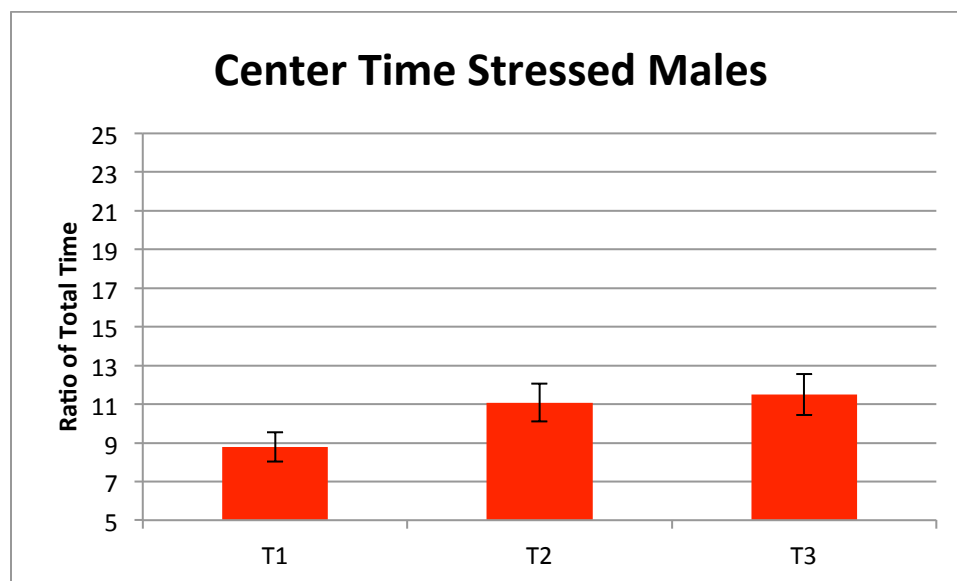
**Figure 20. Center Time, All – Experiment 1.**



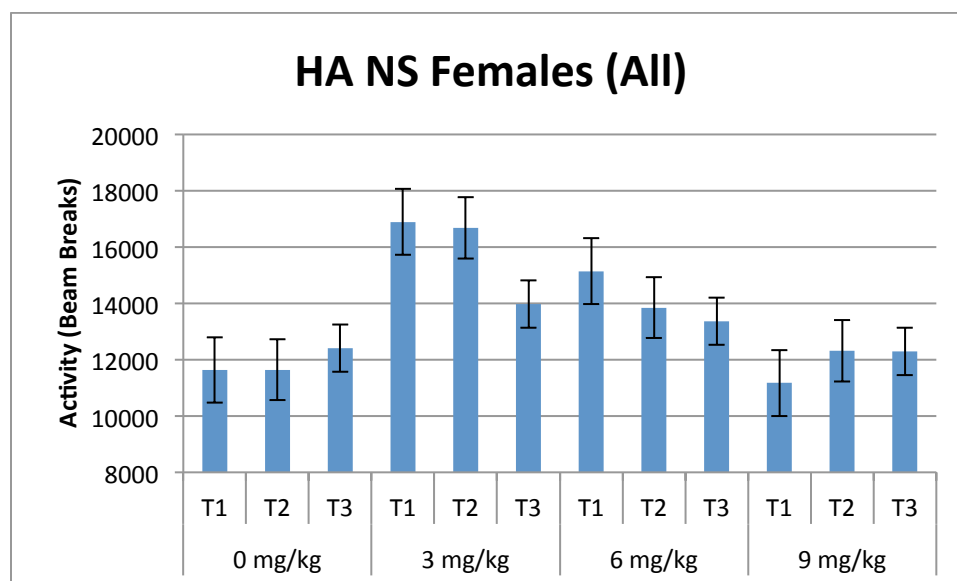
**Figure 21. Center Time, Females – Experiment 1.**



**Figure 22. Center Time, Males – Experiment 1.**



**Figure 23. Center Time, Stressed Males – Experiment 1.**



**Figure 24. Horizontal Activity, Unstressed Females – Experiments 1&2.**

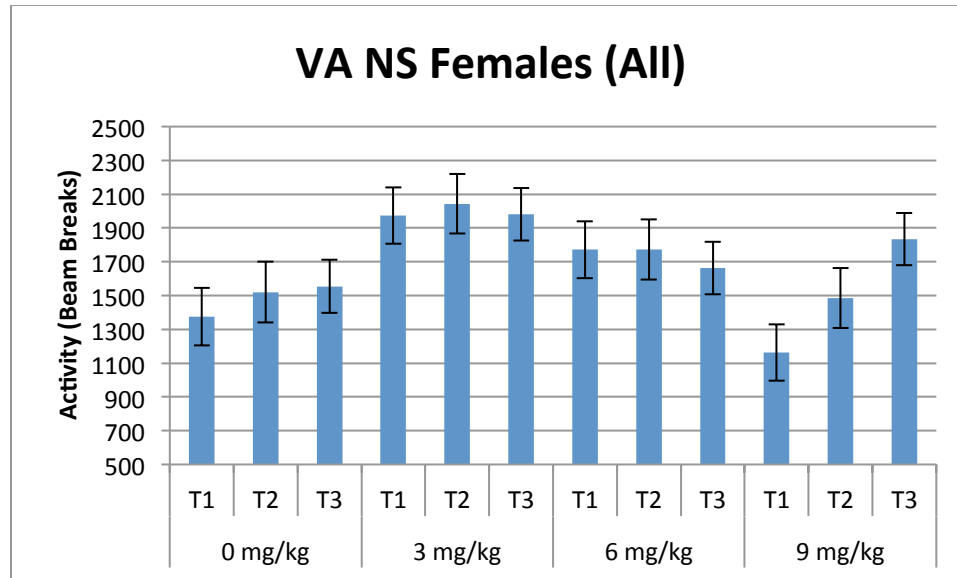


Figure 25. Vertical Activity, Unstressed Females – Experiments 1&2.

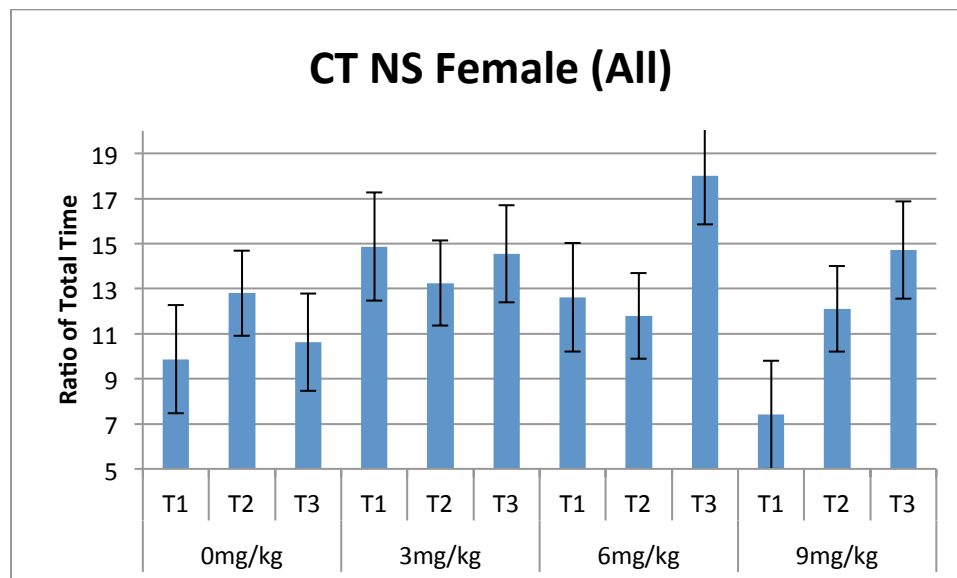


Figure 26. Vertical Activity, Unstressed Females – Experiments 1&2.

## APPENDIX B – TABLES

**Table 1. Treatment cell breakdown, Experiment 1 & 2**

Experiment 1 Subject Breakdown (N=96)		
Sex	Female	48
	Male	48
Stress	No Stress	48
	Stress	48
Nicotine	0 mg/kg	32
	3 mg/kg	32
	6 mg/kg	32

Experiment 2 Subject Breakdown (N=56)		
Sex & Stress	Nicotine	N
Female, Unstressed <i>Includes all Unstressed Females From Experiment 1 &amp; 2</i>	0 mg/kg	14
	3 mg/kg	14
	6 mg/kg	14
	9 mg/kg	14

**Table 2. Overall rANCOVA of Horizontal Activity - Experiment 1**

Overall rANCOVA of Horizontal Activity Within Subject-Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	44513028.888	2	22256514.444	4.406	.014	.091	.713
Time * BLHA	15626840.318	2	7813420.159	1.547	.216	.035	.307
Time * Sex	45526552.490	2	22763276.245	4.506	.012	.086	.686
Time * Stress	3656296.270	2	1828148.135	.362	.697	.009	.111
Time * NIC	87445375.524	4	21861343.881	4.327	.002	.077	.854
Time * Sex * Stress	25618681.937	2	12809340.969	2.536	.082	.052	.445
Time * Sex * NIC	54357786.753	4	13589446.688	2.690	.033	.062	.750
Time * Stress * NIC	3562562.405	4	890640.601	.176	.950	.004	.083
Time * Sex * Stress * NIC	13137653.558	4	3284413.390	.650	.628	.014	.189
Error(Time)	8.386E8	166	5051901.327				

Overall rANCOVA of Horizontal Activity Between Subjects-Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	1.454E9	1	1.454E9	56.864	.000	.407	1.000
BLHA	4.111E8	1	4.111E8	16.072	.000	.162	.977
Sex	1.174E9	1	1.174E9	45.880	.000	.356	1.000
Stress	11799836.446	1	11799836.446	.461	.499	.006	.103
NIC	2.177E8	2	1.088E8	4.256	.017	.093	.729
Sex * Stress	60276263.523	1	60276263.523	2.357	.129	.028	.329
Sex * NIC	46497001.102	2	23248500.551	.909	.407	.021	.202
Stress * NIC	16638440.786	2	8319220.393	.325	.723	.008	.100
Sex * Stress * NIC	42319061.273	2	21159530.636	.827	.441	.020	.187
Error	2.123E9	83	25578217.454				

Table 3. rANCOVA of Horizontal Activity, Females – Experiment 1.

rANCOVA of Horizontal Activity, Female Within Subject-Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	99880632.059	2	49940316.029	9.083	.000	.181	.971
Time * BLHA	55519133.088	2	27759566.544	5.049	.009	.110	.804
Time * Stress	26465373.952	2	13232686.976	2.407	.096	.055	.473
Time * NIC	86209622.849	4	21552405.712	3.920	.006	.161	.886
Time * Stress * NIC	4395680.608	4	1098920.152	.200	.938	.010	.091
Error(Time)	4.509E8	82	5498516.849				

rANCOVA of Horizontal Activity, Female Between Subjects-Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	1.011E9	1	1.011E9	24.366	.000	.373	.998
BLHA	1.717E8	1	1.717E8	4.137	.048	.092	.510
Stress	9857493.030	1	9857493.030	.237	.629	.006	.076
NIC	2.252E8	2	1.126E8	2.712	.078	.117	.507
Stress * NIC	55645859.198	2	27822929.599	.670	.517	.032	.155
Error	1.702E9	41	41509673.555				

**Table 4. rANCOVA of Horizontal Activity, Males - Experiment 1**

rANCOVA of Horizontal Activity, Male Within Subject-Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	194172.851	2	97086.426	.023	.977	.001	.053
Time * BLHA	2480372.549	2	1240186.274	.294	.746	.007	.095
Time * Stress	9396148.365	2	4698074.182	1.115	.333	.026	.240
Time * NIC	47624901.630	4	11906225.408	2.827	.030	.121	.748
Time * Stress * NIC	11844564.838	4	2961141.209	.703	.592	.033	.219
Error(Time)	3.454E8	82	4211763.090				

rANCOVA of Horizontal Activity, Male Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	4.486E8	1	4.486E8	43.797	.000	.516	1.000
BLHA	2.405E8	1	2.405E8	23.481	.000	.364	.997
Stress	63967957.077	1	63967957.077	6.245	.017	.132	.684
NIC	31159280.911	2	15579640.455	1.521	.231	.069	.305
Stress * NIC	3092241.881	2	1546120.940	.151	.860	.007	.072
Error	4.200E8	41	10243193.741				

**Table 5. rANCOVA of Horizontal Activity, Female No Stress – Experiment 1**

rANCOVA of Horizontal Activity, Female No Stress, Within Subject- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	83269839.702	2	41634919.851	7.012	.002	.260	.908
Time * BLHA	44987110.081	2	22493555.041	3.788	.031	.159	.657
Time * NIC	57278097.799	4	14319524.450	2.412	.065	.194	.640
Error(Time)	2.375E8	40	5937706.817				

rANCOVA of Horizontal Activity, Female No Stress, Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	7.894E8	1	7.894E8	22.577	.000	.530	.995
BLHA	52738531.937	1	52738531.937	1.508	.234	.070	.216
NIC	2.154E8	2	1.077E8	3.080	.068	.235	.528
Error	6.993E8	20	34964890.441				

**Table 6. rANCOVA of Horizontal Activity, Female Stress – Experiment 1**

rANCOVA of Horizontal Activity, Female Stress, Within Subject- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time Greenhouse-Geisser	26548721.869	1.374	19318903.710	2.541	.113	.113	.389
Time * BLHA Greenhouse-Geisser	14929716.709	1.374	10864016.767	1.429	.252	.067	.239
Time * NIC Greenhouse-Geisser	33328533.351	2.748	12126209.499	1.595	.216	.138	.357
Error(Time) Greenhouse-Geisser	2.090E8	27.485	7603224.719				

rANCOVA of Horizontal Activity, Female Stress, Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	2.165E8	1	2.165E8	4.473	.047	.183	.521
BLHA	1.534E8	1	1.534E8	3.169	.090	.137	.396
NIC	48614033.044	2	24307016.522	.502	.613	.048	.121
Error	9.682E8	20	48407730.799				

**Table 7. rANCOVA of Horizontal Activity, Male No Stress – Experiment 1.**

rANCOVA of Horizontal Activity, Male No Stress, Within Subject- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	2704581.674	2	1352290.837	.569	.571	.028	.138
Time * BLHA	3730594.749	2	1865297.374	.785	.463	.038	.174
Time * NIC	8677913.114	4	2169478.279	.913	.466	.084	.263
Error(Time)	95080427.168	40	2377010.679				

rANCOVA of Horizontal Activity, Male No Stress, Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power <sup>a</sup>
Intercept	2.419E8	1	2.419E8	18.803	.000	.485	.985
BLHA	84357121.995	1	84357121.995	6.557	.019	.247	.683
NIC	11183256.959	2	5591628.480	.435	.653	.042	.111
Error	2.573E8	20	12864899.023				

**Table 8. rANCOVA of Horizontal Activity, Male Stress – Experiment 1.**

rANCOVA of Horizontal Activity, Male Stress, Within Subject- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	1260001.037	2	630000.518	.101	.005	.005	.064
Time * BLHA	50664.739	2	25332.369	.004	.000	.000	.051
Time * NIC	50389681.685	4	12597420.421	2.024	.168	.168	.554
Error(Time)	2.490E8	40	6224581.482				

rANCOVA of Horizontal Activity, Male Stress, Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	1.990E8	1	1.990E8	26.263	.000	.568	.998
BLHA	1.673E8	1	1.673E8	22.087	.000	.525	.994
NIC	23433036.598	2	11716518.299	1.547	.237	.134	.289
Error	1.515E8	20	7575576.886				

**Table 9. Overall rANCOVA of Vertical Activity – Experiment 1**

Overall rANCOVA of Vertical Activity Within Subject- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	96447.694	2	48223.847	.315	.730	.004	.100
Time * BLVA	297339.305	2	148669.653	.971	.381	.012	.217
Time * Sex	1167605.181	2	583802.590	3.814	.024	.044	.687
Time * Stress	247038.468	2	123519.234	.807	.448	.010	.186
Time * NIC	743736.187	4	185934.047	1.215	.307	.028	.375
Time * Sex * Stress	998626.551	2	499313.276	3.262	.041	.038	.614
Time * Sex * NIC	838304.796	4	209576.199	1.369	.247	.032	.420
Time * Stress * NIC	92445.453	4	23111.363	.151	.962	.004	.081
Time * Sex * Stress * NIC	190064.541	4	47516.135	.310	.871	.007	.118
Error(Time)	25412370.778	166	153086.571				

Overall rANCOVA of Vertical Activity Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	34649594.286	1	34649594.286	70.283	.000	.459	1.000
BLVA	13869104.219	1	13869104.219	28.132	.000	.253	.999
Sex	11636780.446	1	11636780.446	23.604	.000	.221	.998
Stress	1321111.151	1	1321111.151	2.680	.105	.031	.366
NIC	1758823.756	2	879411.878	1.784	.174	.041	.363
Sex * Stress	856851.822	1	856851.822	1.738	.191	.021	.256
Sex * NIC	1632403.834	2	816201.917	1.656	.197	.038	.340
Stress * NIC	135132.941	2	67566.470	.137	.872	.003	.070
Sex * Stress * NIC	1160381.601	2	580190.800	1.177	.313	.028	.251
Error	40918860.823	83	492998.323				

**Table 10. rANCOVA of Vertical Activity, Females – Experiment 1**

rANCOVA of Vertical Activity, Female Within Subject- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	99880632.059	2	49940316.029	9.083	.000	.181	.971
Time * BLHA	55519133.088	2	27759566.544	5.049	.009	.110	.804
Time * Stress	26465373.952	2	13232686.976	2.407	.096	.055	.473
Time * NIC	86209622.849	4	21552405.712	3.920	.006	.161	.886
Time * Stress * NIC	4395680.608	4	1098920.152	.200	.938	.010	.091
Error(Time)	4.509E8	82	5498516.849				

rANCOVA of Vertical Activity, Female Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	19140272.459	1	19140272.459	25.632	.000	.385	.999
BLVA	11600857.582	1	11600857.582	15.535	.000	.275	.970
Stress	62349.967	1	62349.967	.083	.774	.002	.059
NIC	2859031.303	2	1429515.652	1.914	.160	.085	.375
Stress * NIC	680946.393	2	340473.196	.456	.637	.022	.119
Error	30616642.710	41	746747.383				

**Table 11. rANCOVA of Vertical Activity, Males – Experiment 1**

rANCOVA of Vertical Activity, Male Within Subject- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	287394.277	2	143697.139	.959	.387	.023	.211
Time * BLVA	84155.571	2	42077.785	.281	.756	.007	.093
Time * Stress	260100.754	2	130050.377	.868	.423	.021	.195
Time * NIC	773835.492	4	193458.873	1.292	.280	.059	.387
Time * Stress * NIC	180211.229	4	45052.807	.301	.877	.014	.114
Error(Time)	12281494.929	82	149774.328				

rANCOVA of Vertical Activity, Male Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	16066742.950	1	16066742.950	70.715	.000	.633	1.000
BLVA	3255096.288	1	3255096.288	14.327	.000	.259	.959
Stress	1990399.930	1	1990399.930	8.760	.005	.176	.824
NIC	355224.515	2	177612.258	.782	.464	.037	.174
Stress * NIC	315425.066	2	157712.533	.694	.505	.033	.159
Error	9315368.462	41	227204.109				

**Table 12. rANCOVA of Vertical Activity, Female No Stress – Experiment 1**

rANCOVA of Vertical Activity, Female No Stress, Within Subject- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	787707.670	2	393853.835	2.653	.083	.117	.497
Time * BLVA	675977.717	2	337988.859	2.277	.116	.102	.436
Time * NIC	365091.375	4	91272.844	.615	.654	.058	.185
Error(Time)	5938635.616	40	148465.890				

rANCOVA of Vertical Activity, Female No Stress, Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	11107206.921	1	11107206.921	23.792	.000	.543	.996
BLVA	7584967.083	1	7584967.083	16.247	.001	.448	.969
NIC	2916069.396	2	1458034.698	3.123	.066	.238	.534
Error	9336977.208	20	466848.860				

**Table 13. rANCOVA of Vertical Activity, Female Stress – Experiment 1**

rANCOVA of Vertical Activity, Female Stress, Within Subject- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	146237.930	2	73118.965	.443	.645	.022	.117
Time * BLVA	125628.545	2	62814.273	.380	.686	.019	.107
Time * NIC	600254.604	4	150063.651	.909	.468	.083	.262
Error(Time)	6603817.705	40	165095.443				

rANCOVA of Vertical Activity, Female Stress, Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	7304871.472	1	7304871.472	6.866	.016	.256	.703
BLVA	4018334.971	1	4018334.971	3.777	.066	.159	.456
NIC	615438.000	2	307719.000	.289	.752	.028	.090
Error	21277221.029	20	1063861.051				

**Table 14. rANCOVA of Vertical Activity, Male No Stress – Experiment 1**

rANCOVA of Vertical Activity, Male No Stress, Within Subject- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	154246.037	2	77123.019	.781	.465	.038	.174
Time * BLVA	14204.344	2	7102.172	.072	.931	.004	.060
Time * NIC	161056.884	4	40264.221	.408	.802	.039	.134
Error(Time)	3950534.489	40	98763.362				

rANCOVA of Vertical Activity, Male No Stress, Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	7300024.721	1	7300024.721	30.644	.000	.605	1.000
BLVA	1514674.334	1	1514674.334	6.358	.020	.241	.670
NIC	571990.059	2	285995.029	1.201	.322	.107	.232
Error	4764442.958	20	238222.148				

**Table 15. rANCOVA of Vertical Activity, Male Stress – Experiment 1**

rANCOVA of Vertical Activity, Male Stress, Within Subject- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	423807.988	2	211903.994	1.051	.359	.050	.221
Time * BLVA	332390.080	2	166195.040	.824	.446	.040	.181
Time * NIC	775364.592	4	193841.148	.961	.439	.088	.276
Error(Time)	8068521.587	40	201713.040				

rANCOVA of Vertical Activity, Male Stress, Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	8456372.873	1	8456372.873	37.468	.000	.652	1.000
BLVA	1777447.657	1	1777447.657	7.875	.011	.283	.761
NIC	30796.547	2	15398.274	.068	.934	.007	.059
Error	4513899.801	20	225694.990				

**Table 16. Overall rANCOVA of Center Time – Experiment 1**

rANCOVA of Center Time Within Subject- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	302.204	2	151.102	6.107	.003	.069	.883
Time * BLRt	87.680	2	43.840	1.772	.173	.021	.367
Time * Sex	21.497	2	10.748	.434	.648	.005	.120
Time * Stress	26.064	2	13.032	.527	.592	.006	.136
Time * NIC	62.529	4	15.632	.632	.640	.015	.204
Time * Sex * Stress	80.084	2	40.042	1.618	.201	.019	.339
Time * Sex * NIC	27.185	4	6.796	.275	.894	.007	.110
Time * Stress * NIC	225.030	4	56.257	2.274	.063	.052	.654
Time * Sex * Stress * NIC	113.329	4	28.332	1.145	.337	.027	.355
Error(Time)	4107.083	166	24.741				

rANCOVA of Center Time Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	8041.488	1	8041.488	92.452	.000	.527	1.000
BLRt	146.818	1	146.818	1.688	.197	.020	.250
Sex	874.031	1	874.031	10.049	.002	.108	.880
Stress	100.923	1	100.923	1.160	.285	.014	.187
NIC	255.132	2	127.566	1.467	.237	.034	.305
Sex * Stress	132.025	1	132.025	1.518	.221	.018	.230
Sex * NIC	12.513	2	6.257	.072	.931	.002	.061
Stress * NIC	251.693	2	125.846	1.447	.241	.034	.302
Sex * Stress * NIC	147.813	2	73.907	.850	.431	.020	.191
Error	7219.322	83	86.980				

**Table 17. rANCOVA of Center Time, Females – Experiment 1**

rANCOVA of Center Time Female, Within Subject- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	159.033	2	79.516	2.332	.104	.054	.460
Time * BLRt	65.297	2	32.649	.958	.388	.023	.211
Time * Stress	98.694	2	49.347	1.447	.241	.034	.301
Time * NIC	33.053	4	8.263	.242	.914	.012	.100
Time * Stress * NIC	284.979	4	71.245	2.089	.090	.092	.598
Error(Time)	2795.956	82	34.097				

rANCOVA of Center Time Female, Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	6375.799	1	6375.799	47.408	.000	.536	1.000
BLRt	72.143	1	72.143	.536	.468	.013	.110
Stress	227.031	1	227.031	1.688	.201	.040	.245
NIC	139.736	2	69.868	.520	.599	.025	.130
Stress * NIC	263.895	2	131.948	.981	.384	.046	.209
Error	5513.957	41	134.487				

**Table 18. rANCOVA of Center Time, Males – Experiment 1**

rANCOVA of Center Time Male, Within Subject- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	125.619	2	62.810	3.929	.023	.087	.692
Time * BLRt	22.624	2	11.312	.708	.496	.017	.166
Time * Stress	7.368	2	3.684	.230	.795	.006	.085
Time * NIC	56.441	4	14.110	.883	.478	.041	.269
Time * Stress * NIC	50.897	4	12.724	.796	.531	.037	.245
Error(Time)	1310.886	82	15.986				

rANCOVA of Center Time Male, Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	1885.050	1	1885.050	45.635	.000	.527	1.000
BLRt	86.435	1	86.435	2.092	.156	.049	.292
Stress	.256	1	.256	.006	.938	.000	.051
NIC	136.793	2	68.396	1.656	.203	.075	.329
Stress * NIC	130.002	2	65.001	1.574	.220	.071	.314
Error	1693.604	41	41.307				

**Table 19. rANCOVA of Center Time, Female No Stress – Experiment 1**

rANCOVA of Center Time, Female, Unstressed, Within Subject- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	131.968	2	65.984	1.377	.264	.064	.279
Time * BLRt	135.405	2	67.703	1.413	.255	.066	.285
Time * NIC	202.032	4	50.508	1.054	.392	.095	.301
Error(Time)	1916.463	40	47.912				

rANCOVA of Center Time, Female, Unstressed, Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	4923.880	1	4923.880	21.569	.000	.519	.993
BLRt	76.242	1	76.242	.334	.570	.016	.085
NIC	368.780	2	184.390	.808	.460	.075	.168
Error	4565.759	20	228.288				

**Table 20. rANCOVA of Center Time, Female Stress – Experiment 1**

rANCOVA of Center Time, Female, Stressed, Within Subject- Experiment 1

		Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Source								
Time	Greenhouse-Geisser	112.247	1.525	73.595	2.883	.084	.126	.459
Time * BLRt	Greenhouse-Geisser	30.699	1.525	20.128	.788	.432	.038	.157
Time * NIC	Greenhouse-Geisser	107.916	3.050	35.378	1.386	.266	.122	.333
Error(Time)	Greenhouse-Geisser	778.687	30.504	25.527				

rANCOVA of Center Time, Female, Stressed, Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	1944.012	1	1944.012	61.165	.000	.754	1.000
BLRt	308.443	1	308.443	9.705	.005	.327	.842
NIC	148.705	2	74.353	2.339	.122	.190	.418
Error	635.657	20	31.783				

**Table 21. rANCOVA of Center Time, Male No Stress – Experiment 1**

rANCOVA of Center Time, Male, Unstressed, Within Subject- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	6.356	2	3.178	.194	.824	.010	.078
Time * BLRt	12.620	2	6.310	.385	.683	.019	.108
Time * NIC	48.417	4	12.104	.739	.571	.069	.217
Error(Time)	654.922	40	16.373				

rANCOVA of Center Time, Male, Unstressed, Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	317.544	1	317.544	7.998	.010	.286	.767
BLRt	300.964	1	300.964	7.580	.012	.275	.745
NIC	63.681	2	31.840	.802	.462	.074	.168
Error	794.104	20	39.705				

**Table 22. rANCOVA of Center Time, Male Stress – Experiment 1**

rANCOVA of Center Time, Male, Stressed, Within Subject- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	136.971	2	68.485	4.595	.016	.187	.746
Time * BLRt	69.801	2	34.900	2.342	.109	.105	.447
Time * NIC	65.777	4	16.444	1.103	.368	.099	.315
Error(Time)	596.168	40	14.904				

rANCOVA of Center Time, Male, Stressed, Between Subjects- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	1606.472	1	1606.472	47.062	.000	.702	1.000
BLRt	2.266	1	2.266	.066	.799	.003	.057
NIC	166.568	2	83.284	2.440	.113	.196	.433
Error	682.705	20	34.135				

**Table 23. rANCOVA of Horizontal Activity, Female No Stress – Experiments 1 & 2**

rANCOVA of Horizontal Activity, Female Unstressed, Within Subjects- Experiment 1 & 2

Source		Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	Greenhouse-Geisser	18263868.921	1.739	10500930.044	1.390	.254	.027	.273
Time * BLHA	Greenhouse-Geisser	11177386.283	1.739	6426510.831	.850	.417	.016	.182
Time * NIC	Greenhouse-Geisser	97758248.751	5.218	18735579.990	2.479	.036	.127	.767
Error(Time)	Greenhouse-Geisser	6.703E8	88.702	7556859.513				

rANCOVA of Horizontal Activity, Female Unstressed, Between Subjects- Experiment 1 & 2

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	1.573E9	1	1.573E9	49.083	.000	.490	1.000
BLHA	3.137E8	1	3.137E8	9.787	.003	.161	.866
NIC	4.541E8	3	1.514E8	4.722	.006	.217	.874
Error	1.635E9	51	32055571.554				

**Table 24. rANCOVA of Vertical Activity, Female No Stress – Experiment 1 & 2**

rANCOVA of Vertical Activity, Female Unstressed, Within Subject- Experiment 1 & 2

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	321225.624	2	160612.812	.804	.450	.016	.184
Time * BLVA	643153.875	2	321576.937	1.609	.205	.031	.334
Time * NIC	2523994.882	6	420665.814	2.105	.059	.110	.733
Error(Time)	20381865.078	102	199822.207				

rANCOVA of Vertical Activity, Female Unstressed, Between Subjects- Experiment 1 & 2

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	31421241.244	1	31421241.244	40.648	.000	.444	1.000
BLVA	11728345.161	1	11728345.161	15.172	.000	.229	.969
NIC	7445092.672	3	2481697.557	3.210	.031	.159	.707
Error	39423653.101	51	773012.806				

**Table 25. rANCOVA of Center Time, Female No Stress – Experiment 1 & 2**

rANCOVA of Center Time, Female Unstressed, Within Subject- Experiment 1 & 2

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	378.636	2	189.318	5.006	.008	.089	.804
Time * BLRt	161.324	2	80.662	2.133	.124	.040	.428
Time * NIC	478.814	6	79.802	2.110	.058	.110	.734
Error(Time)	3857.193	102	37.816				

rANCOVA of Center Time, Female Unstressed, Between Subject- Experiment 1 & 2

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	5408.177	1	5408.177	45.104	.000	.469	1.000
BLRt	67.442	1	67.442	.562	.457	.011	.114
NIC	355.953	3	118.651	.990	.405	.055	.254
Error	6115.194	51	119.906				

**Table 26. ANCOVA of Horizontal Activity, Female No Stress, T1 – Experiment 1**

ANCOVA of Horizontal Activity, Female Unstressed, T1- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	1.562E8	3	52057511.123	3.948	.023	.372	.749
Intercept	5.485E8	1	5.485E8	41.604	.000	.675	1.000
BLHA	1543896.120	1	1543896.120	.117	.736	.006	.062
NIC	1.461E8	2	73029639.082	5.539	.012	.356	.794
Error	2.637E8	20	13184449.913				
Total	6.893E9	24					
Corrected Total	4.199E8	23					

**Table 27. ANCOVA of Horizontal Activity, Female Stress, T1 – Experiment 1**

ANCOVA of Horizontal Activity, Female Stressed, T1- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	50950431.910 <sup>a</sup>	3	16983477.303	1.045	.394	.136	.241
Intercept	1.557E8	1	1.557E8	9.582	.006	.324	.837
BLHA	18517030.327	1	18517030.327	1.140	.298	.054	.174
NIC	35254728.645	2	17627364.323	1.085	.357	.098	.213
Error	3.250E8	20	16247814.696				
Total	5.762E9	24					
Corrected Total	3.759E8	23					

**Table 28. ANCOVA of Horizontal Activity, Male No Stress, T1 – Experiment 1**

ANCOVA of Horizontal Activity, Male Unstressed, T1-Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	70474485.017 <sup>a</sup>	3	23491495.006	3.657	.030	.354	.712
Intercept	58848406.470	1	58848406.470	9.161	.007	.314	.821
BLHA	47215635.767	1	47215635.767	7.350	.013	.269	.732
NIC	16299909.451	2	8149954.726	1.269	.303	.113	.243
Error	1.285E8	20	6423492.730				
Total	2.288E9	24					
Corrected Total	1.989E8	23					

**Table 29. ANCOVA of Horizontal Activity, Male Stress, T1 – Experiment 1**

ANCOVA of Horizontal Activity, Male Stressed, T1- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	1.213E8	3	40424853.809	7.388	.002	.526	.960
Intercept	82080619.973	1	82080619.973	15.000	.001	.429	.957
BLHA	58547591.177	1	58547591.177	10.700	.004	.349	.875
NIC	60709751.200	2	30354875.600	5.547	.012	.357	.795
Error	1.094E8	20	5471935.229				
Total	3.166E9	24					
Corrected Total	2.307E8	23					

**Table 30. ANCOVA of Horizontal Activity, Female No Stress, T2 – Experiment 1**

ANCOVA of Horizontal Activity, Female Unstressed, T2 – Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	1.364E8	3	45480999.428	2.085	.134	.238	.453
Intercept	2.040E8	1	2.040E8	9.352	.006	.319	.829
BLHA	40074085.951	1	40074085.951	1.837	.190	.084	.252
NIC	1.146E8	2	57301237.573	2.627	.097	.208	.462
Error	4.363E8	20	21815340.377				
Total	6.009E9	24					
Corrected Total	5.727E8	23					

**Table 31. ANCOVA of Horizontal Activity, Female Stress, T2 – Experiment 1**

ANCOVA of Horizontal Activity, Female Stressed, T2- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	1.301E8	3	43380108.031	2.183	.122	.247	.472
Intercept	28397171.499	1	28397171.499	1.429	.246	.067	.207
BLHA	95072819.759	1	95072819.759	4.785	.041	.193	.549
NIC	44583980.672	2	22291990.336	1.122	.345	.101	.219
Error	3.974E8	20	19870150.693				
Total	4.788E9	24					
Corrected Total	5.275E8	23					

**Table 32. ANCOVA of Horizontal Activity, Male No Stress, T2 – Experiment 1**

ANCOVA of Horizontal Activity, Male Unstressed, T2- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	19990375.092 <sup>a</sup>	3	6663458.364	1.078	.381	.139	.247
Intercept	87840203.794	1	87840203.794	14.212	.001	.415	.948
BLHA	19162507.508	1	19162507.508	3.100	.094	.134	.388
NIC	73443.345	2	36721.672	.006	.994	.001	.051
Error	1.236E8	20	6180808.937				
Total	2.040E9	24					
Corrected Total	1.436E8	23					

**Table 33. ANCOVA of Horizontal Activity, Male Stress, T2 – Experiment 1**

ANCOVA of Horizontal Activity, Male Stressed, T2- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power <sup>b</sup>
Corrected Model	56144846.279 <sup>a</sup>	3	18714948.760	2.654	.076	.285	.559
Intercept	59400978.610	1	59400978.610	8.425	.009	.296	.788
BLHA	54554453.945	1	54554453.945	7.738	.012	.279	.754
NIC	859045.588	2	429522.794	.061	.941	.006	.058
Error	1.410E8	20	7050518.034				
Total	2.584E9	24					
Corrected Total	1.972E8	23					

**Table 34. ANCOVA of Horizontal Activity, Female No Stress, T3 – Experiment 1**

ANCOVA of Horizontal Activity, Female Unstressed, T3- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	60032880.280 <sup>a</sup>	3	20010960.093	1.690	.201	.202	.374
Intercept	1.201E8	1	1.201E8	10.145	.005	.337	.857
BLHA	56107659.947	1	56107659.947	4.739	.042	.192	.545
NIC	11993464.887	2	5996732.443	.506	.610	.048	.122
Error	2.368E8	20	11840513.784				
Total	4.618E9	24					
Corrected Total	2.968E8	23					

**Table 35. ANCOVA of Horizontal Activity, Female Stress, T3 – Experiment 1**

ANCOVA of Horizontal Activity, Female Stressed, T3- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	65021023.093 <sup>a</sup>	3	21673674.364	.953	.434	.125	.222
Intercept	58998985.113	1	58998985.113	2.595	.123	.115	.335
BLHA	54756075.093	1	54756075.093	2.408	.136	.107	.315
NIC	2103857.078	2	1051928.539	.046	.955	.005	.056
Error	4.548E8	20	22738386.170				
Total	4.817E9	24					
Corrected Total	5.198E8	23					

**Table 36. ANCOVA of Horizontal Activity, Male No Stress, T3 – Experiment 1**

ANCOVA of Horizontal Activity, Male Unstressed, T3- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	27693333.718 <sup>a</sup>	3	9231111.239	1.841	.172	.216	.405
Intercept	97918369.141	1	97918369.141	19.527	.000	.494	.987
BLHA	21709573.468	1	21709573.468	4.329	.051	.178	.508
NIC	3487817.277	2	1743908.639	.348	.710	.034	.098
Error	1.003E8	20	5014618.714				
Total	2.253E9	24					
Corrected Total	1.280E8	23					

**Table 37. ANCOVA of Horizontal Activity, Male Stress, T3 – Experiment 1**

ANCOVA of Horizontal Activity, Male Stressed, T3- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	70552707.236 <sup>a</sup>	3	23517569.079	3.135	.048	.320	.638
Intercept	58736016.913	1	58736016.913	7.829	.011	.281	.759
BLHA	54270323.153	1	54270323.153	7.234	.014	.266	.725
NIC	12253921.494	2	6126960.747	.817	.456	.076	.170
Error	1.500E8	20	7502286.586				
Total	2.588E9	24					
Corrected Total	2.206E8	23					

**Table 38. ANCOVA of Vertical Activity, Female No Stress, T1 – Experiment 1**

ANCOVA of Vertical Activity, Female Unstressed, T1- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	2887674.909 <sup>a</sup>	3	962558.303	3.496	.035	.344	.691
Intercept	6841258.265	1	6841258.265	24.849	.000	.554	.997
BLVA	847349.325	1	847349.325	3.078	.095	.133	.386
NIC	1763139.407	2	881569.703	3.202	.062	.243	.545
Error	5506214.050	20	275310.702				
Total	97973001.000	24					
Corrected Total	8393888.958	23					

**Table 39. ANCOVA of Vertical Activity, Female Stress, T1 – Experiment 1**

ANCOVA of Vertical Activity, Female Stressed, T1- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	1161115.514 <sup>a</sup>	3	387038.505	1.094	.375	.141	.251
Intercept	3458339.999	1	3458339.999	9.774	.005	.328	.845
BLVA	757063.180	1	757063.180	2.140	.159	.097	.286
NIC	451909.425	2	225954.713	.639	.538	.060	.142
Error	7076552.445	20	353827.622				
Total	92864105.000	24					
Corrected Total	8237667.958	23					

**Table 40. ANCOVA of Vertical Activity, Female Stress, T1 – Experiment 1**

ANCOVA of Vertical Activity, Male Unstressed, T1- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	851211.575 <sup>a</sup>	3	283737.192	1.812	.178	.214	.399
Intercept	1734097.753	1	1734097.753	11.072	.003	.356	.886
BLVA	428933.991	1	428933.991	2.739	.114	.120	.351
NIC	562412.092	2	281206.046	1.795	.192	.152	.330
Error	3132486.384	20	156624.319				
Total	36122845.000	24					
Corrected Total	3983697.958	23					

**Table 41. ANCOVA of Vertical Activity, Male Stress, T1 – Experiment 1**

ANCOVA of Vertical Activity, Male Stressed, T1- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	1636759.500 <sup>a</sup>	3	545586.500	3.185	.046	.323	.646
Intercept	1663199.795	1	1663199.795	9.710	.005	.327	.842
BLVA	1083549.416	1	1083549.416	6.326	.021	.240	.668
NIC	505177.996	2	252588.998	1.475	.253	.129	.277
Error	3425824.459	20	171291.223				
Total	56329081.000	24					
Corrected Total	5062583.958	23					

**Table 42. ANCOVA of Vertical Activity, Female No Stress, T2 – Experiment 1**

ANCOVA of Vertical Activity, Female Unstressed, T2- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	5588060.006 <sup>a</sup>	3	1862686.669	6.014	.004	.474	.913
Intercept	3120046.218	1	3120046.218	10.074	.005	.335	.855
BLVA	3868384.422	1	3868384.422	12.490	.002	.384	.919
NIC	1250337.224	2	625168.612	2.019	.159	.168	.367
Error	6194350.953	20	309717.548				
Total	1.079E8	24					
Corrected Total	11782410.958	23					

**Table 43. ANCOVA of Vertical Activity, Female Stress, T2 – Experiment 1**

ANCOVA of Vertical Activity, Female Stressed, T2- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	2347968.296 <sup>a</sup>	3	782656.099	1.383	.277	.172	.311
Intercept	1778429.110	1	1778429.110	3.144	.091	.136	.393
BLVA	1614111.046	1	1614111.046	2.853	.107	.125	.363
NIC	706524.177	2	353262.089	.624	.546	.059	.140
Error	11314924.329	20	565746.216				
Total	89523041.000	24					
Corrected Total	13662892.625	23					

**Table 44. ANCOVA of Vertical Activity, Male No Stress, T2 – Experiment 1**

ANCOVA of Vertical Activity, Male Unstressed, T2- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	674531.459 <sup>a</sup>	3	224843.820	1.127	.362	.145	.257
Intercept	2250925.099	1	2250925.099	11.280	.003	.361	.891
BLVA	652085.876	1	652085.876	3.268	.086	.140	.406
NIC	86063.584	2	43031.792	.216	.808	.021	.079
Error	3991001.499	20	199550.075				
Total	48597443.000	24					
Corrected Total	4665532.958	23					

**Table 45. ANCOVA of Vertical Activity, Male Stress, T2 – Experiment 1**

ANCOVA of Vertical Activity, Male Stressed, T2- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	189790.370 <sup>a</sup>	3	63263.457	.362	.781	.052	.109
Intercept	4783039.681	1	4783039.681	27.377	.000	.578	.999
BLVA	90549.037	1	90549.037	.518	.480	.025	.105
NIC	87126.715	2	43563.358	.249	.782	.024	.084
Error	3494233.588	20	174711.679				
Total	64145551.000	24					
Corrected Total	3684023.958	23					

**Table 46. ANCOVA of Vertical Activity, Female No Stress, T3 – Experiment 1**

ANCOVA of Vertical Activity, Female Unstressed, T3- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	4051796.136 <sup>a</sup>	3	1350598.712	7.556	.001	.531	.964
Intercept	1933610.109	1	1933610.109	10.817	.004	.351	.878
BLVA	3545211.053	1	3545211.053	19.833	.000	.498	.988
NIC	267684.140	2	133842.070	.749	.486	.070	.159
Error	3575047.822	20	178752.391				
Total	80997401.000	24					
Corrected Total	7626843.958	23					

**Table 47. ANCOVA of Vertical Activity, Female Stress, T3 – Experiment 1**

ANCOVA of Vertical Activity, Female Stressed, T3- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	1904015.373 <sup>a</sup>	3	634671.791	1.338	.290	.167	.301
Intercept	2214340.293	1	2214340.293	4.667	.043	.189	.538
BLVA	1772789.290	1	1772789.290	3.736	.068	.157	.452
NIC	57259.002	2	28629.501	.060	.942	.006	.058
Error	9489561.960	20	474478.098				
Total	1.005E8	24					
Corrected Total	11393577.333	23					

**Table 48. ANCOVA of Vertical Activity, Male No Stress, T3 – Experiment 1**

ANCOVA of Vertical Activity, Male Unstressed, T3- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	471329.394 <sup>a</sup>	3	157109.798	1.974	.150	.228	.431
Intercept	3469247.906	1	3469247.906	43.597	.000	.686	1.000
BLVA	447858.811	1	447858.811	5.628	.028	.220	.617
NIC	84571.268	2	42285.634	.531	.596	.050	.125
Error	1591489.564	20	79574.478				
Total	55491339.000	24					
Corrected Total	2062818.958	23					

**Table 49. ANCOVA of Vertical Activity, Male Stress, T3 – Experiment 1**

ANCOVA of Vertical Activity, Male Stressed, T3- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	1062972.284 <sup>a</sup>	3	354324.095	1.252	.318	.158	.283
Intercept	2433941.386	1	2433941.386	8.597	.008	.301	.796
BLVA	935739.284	1	935739.284	3.305	.084	.142	.409
NIC	213856.428	2	106928.214	.378	.690	.036	.102
Error	5662363.341	20	283118.167				
Total	67428359.000	24					
Corrected Total	6725335.625	23					

**Table 50. ANCOVA of Center Time, Female No Stress, T1 – Experiment 1**

ANCOVA of Center Time, Female Unstressed, T1- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	244.600 <sup>a</sup>	3	81.533	.504	.684	.070	.134
Intercept	979.660	1	979.660	6.057	.023	.232	.649
BLRt	17.147	1	17.147	.106	.748	.005	.061
NIC	198.530	2	99.265	.614	.551	.058	.138
Error	3234.575	20	161.729				
Total	8756.016	24					
Corrected Total	3479.175	23					

**Table 51. ANCOVA of Center Time, Female Stress, T1 – Experiment 1**

ANCOVA of Center Time, Female Stressed, T1- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	76.705 <sup>a</sup>	3	25.568	1.284	.307	.161	.290
Intercept	502.450	1	502.450	25.230	.000	.558	.998
BLRt	74.599	1	74.599	3.746	.067	.158	.453
NIC	5.030	2	2.515	.126	.882	.012	.067
Error	398.293	20	19.915				
Total	3530.519	24					
Corrected Total	474.998	23					

**Table 52. ANCOVA of Center Time, Male No Stress, T1 – Experiment 1**

ANCOVA of Center Time, Male Unstressed, T1- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	104.731 <sup>a</sup>	3	34.910	2.558	.084	.277	.542
Intercept	75.797	1	75.797	5.554	.029	.217	.611
BLRt	51.004	1	51.004	3.737	.067	.157	.452
NIC	45.695	2	22.847	1.674	.213	.143	.310
Error	272.930	20	13.646				
Total	1885.626	24					
Corrected Total	377.660	23					

**Table 53. ANCOVA of Center Time, Male Stress, T1 – Experiment 1**

ANCOVA of Center Time, Male Stressed, T1- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	80.916 <sup>a</sup>	3	26.972	1.986	.148	.230	.434
Intercept	204.957	1	204.957	15.095	.001	.430	.958
BLRt	24.733	1	24.733	1.822	.192	.083	.251
NIC	63.161	2	31.580	2.326	.124	.189	.416
Error	271.556	20	13.578				
Total	2204.498	24					
Corrected Total	352.472	23					

**Table 54. ANCOVA of Center Time, Female No Stress, T2 – Experiment 1**

ANCOVA of Center Time, Female Unstressed, T2- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	73.094 <sup>a</sup>	3	24.365	.412	.746	.058	.117
Intercept	1900.661	1	1900.661	32.126	.000	.616	1.000
BLRt	56.524	1	56.524	.955	.340	.046	.154
NIC	27.884	2	13.942	.236	.792	.023	.082
Error	1183.263	20	59.163				
Total	7178.542	24					
Corrected Total	1256.357	23					

**Table 55. ANCOVA of Center Time, Female Stress, T2 – Experiment 1**

ANCOVA of Center Time, Female Stressed, T2- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	279.105 <sup>a</sup>	3	93.035	4.479	.015	.402	.805
Intercept	398.506	1	398.506	19.184	.000	.490	.986
BLRt	212.764	1	212.764	10.242	.004	.339	.861
NIC	83.057	2	41.529	1.999	.162	.167	.363
Error	415.457	20	20.773				
Total	4300.620	24					
Corrected Total	694.562	23					

**Table 56. ANCOVA of Center Time, Male No Stress, T2 – Experiment 1**

ANCOVA of Center Time, Male Unstressed, T2- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	138.972 <sup>a</sup>	3	46.324	2.250	.114	.252	.485
Intercept	98.778	1	98.778	4.798	.041	.193	.550
BLRt	123.579	1	123.579	6.002	.024	.231	.645
NIC	16.061	2	8.031	.390	.682	.038	.104
Error	411.775	20	20.589				
Total	3178.231	24					
Corrected Total	550.747	23					

**Table 57. ANCOVA of Center Time, Male Stress, T2 – Experiment 1**

ANCOVA of Center Time, Male Stressed, T2- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	150.324 <sup>a</sup>	3	50.108	2.144	.127	.243	.465
Intercept	594.260	1	594.260	25.428	.000	.560	.998
BLRt	.549	1	.549	.023	.880	.001	.052
NIC	145.064	2	72.532	3.104	.067	.237	.532
Error	467.399	20	23.370				
Total	3566.723	24					
Corrected Total	617.722	23					

**Table 58. ANCOVA of Center Time, Female No Stress, T3 – Experiment 1**

ANCOVA of Center Time, Female Unstressed, T3- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	437.896 <sup>a</sup>	3	145.965	1.414	.268	.175	.317
Intercept	2175.526	1	2175.526	21.077	.000	.513	.992
BLRt	137.976	1	137.976	1.337	.261	.063	.196
NIC	344.398	2	172.199	1.668	.214	.143	.309
Error	2064.383	20	103.219				
Total	8214.705	24					
Corrected Total	2502.280	23					

**Table 59. ANCOVA of Center Time, Female Stress, T3 – Experiment 1**

ANCOVA of Center Time, Female Stressed, T3- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	194.286 <sup>a</sup>	3	64.762	2.157	.125	.244	.467
Intercept	1155.303	1	1155.303	38.472	.000	.658	1.000
BLRt	51.778	1	51.778	1.724	.204	.079	.240
NIC	168.534	2	84.267	2.806	.084	.219	.489
Error	600.594	20	30.030				
Total	6353.490	24					
Corrected Total	794.879	23					

**Table 60. ANCOVA of Center Time, Male No Stress, T3 – Experiment 1**

ANCOVA of Center Time, Male Unstressed, T3- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	202.666 <sup>a</sup>	3	67.555	1.768	.186	.210	.390
Intercept	149.326	1	149.326	3.907	.062	.163	.469
BLRt	139.001	1	139.001	3.637	.071	.154	.443
NIC	50.341	2	25.171	.659	.528	.062	.145
Error	764.321	20	38.216				
Total	4404.849	24					
Corrected Total	966.987	23					

**Table 61. ANCOVA of Center Time, Male Stress, T3 – Experiment 1**

ANCOVA of Center Time, Male Stressed, T3- Experiment 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	64.558 <sup>a</sup>	3	21.519	.797	.510	.107	.191
Intercept	944.227	1	944.227	34.977	.000	.636	1.000
BLRt	46.786	1	46.786	1.733	.203	.080	.241
NIC	24.120	2	12.060	.447	.646	.043	.113
Error	539.919	20	26.996				
Total	3772.231	24					
Corrected Total	604.477	23					

**Table 62. ANCOVA of Horizontal Activity, Female No Stress, T1 – Experiment 1&2**

ANCOVA of Horizontal Activity, T1- Experiment 1&amp;2

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	3.495E8	4	87364308.404	4.590	.003	.265	.925
Intercept	6.775E8	1	6.775E8	35.594	.000	.411	1.000
BLHA	57709000.419	1	57709000.419	3.032	.088	.056	.401
NIC	3.168E8	3	1.056E8	5.549	.002	.246	.924
Error	9.707E8	51	19033731.161				
Total	1.185E10	56					
Corrected Total	1.320E9	55					

**Table 63. ANCOVA of Horizontal Activity, Female No Stress, T2 – Experiment 1&2**

ANCOVA of Horizontal Activity, T2- Experiment 1&amp;2

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	3.002E8	4	75043386.558	4.588	.003	.265	.925
Intercept	5.143E8	1	5.143E8	31.443	.000	.381	1.000
BLHA	1.189E8	1	1.189E8	7.272	.009	.125	.754
NIC	2.082E8	3	69403941.618	4.243	.009	.200	.833
Error	8.342E8	51	16356017.880				
Total	1.152E10	56					
Corrected Total	1.134E9	55					

**Table 64. ANCOVA of Horizontal Activity, Female No Stress, T3 – Experiment 1&2**

ANCOVA of Horizontal Activity, T3- Experiment 1&amp;2

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	1.651E8	4	41278346.891	4.208	.005	.248	.899
Intercept	3.999E8	1	3.999E8	40.766	.000	.444	1.000
BLHA	1.482E8	1	1.482E8	15.113	.000	.229	.968
NIC	26790658.150	3	8930219.383	.910	.443	.051	.236
Error	5.003E8	51	9809181.341				
Total	1.014E10	56					
Corrected Total	6.654E8	55					

**Table 65. ANCOVA of Vertical Activity, Female No Stress, T1 – Experiment 1&2**

ANCOVA of Vertical Activity, T1- Experiment 1&amp;2

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	8229777.053 <sup>a</sup>	4	2057444.263	5.242	.001	.291	.956
Intercept	11613793.715	1	11613793.715	29.588	.000	.367	1.000
BLVA	2064046.338	1	2064046.338	5.258	.026	.093	.614
NIC	5682185.547	3	1894061.849	4.825	.005	.221	.882
Error	20018374.376	51	392517.145				
Total	1.663E8	56					
Corrected Total	28248151.429	55					

**Table 66. ANCOVA of Vertical Activity, Female No Stress, T2 – Experiment 1&2**

ANCOVA of Vertical Activity, T2- Experiment 1&amp;2

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	10198070.726 <sup>a</sup>	4	2549517.681	5.756	.001	.311	.971
Intercept	7719094.797	1	7719094.797	17.428	.000	.255	.984
BLVA	6592331.101	1	6592331.101	14.884	.000	.226	.966
NIC	2811491.560	3	937163.853	2.116	.110	.111	.509
Error	22588059.256	51	442903.123				
Total	1.956E8	56					
Corrected Total	32786129.982	55					

**Table 67. ANCOVA of Vertical Activity, Female No Stress, T3 – Experiment 1&2**

ANCOVA of Vertical Activity, T3- Experiment 1&amp;2

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	5706948.311 <sup>a</sup>	4	1426737.078	4.231	.005	.249	.900
Intercept	12409578.356	1	12409578.356	36.798	.000	.419	1.000
BLVA	3715121.597	1	3715121.597	11.016	.002	.178	.903
NIC	1475410.447	3	491803.482	1.458	.237	.079	.363
Error	17199084.546	51	337236.952				
Total	1.959E8	56					
Corrected Total	22906032.857	55					

**Table 68. ANCOVA of Center Time, Female No Stress, T1 – Experiment 1&2**

ANCOVA of Center Time, T1- Experiment 1&amp;2

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	623.488 <sup>a</sup>	4	155.872	1.934	.119	.132	.544
Intercept	987.446	1	987.446	12.252	.001	.194	.930
BLRt	120.595	1	120.595	1.496	.227	.029	.225
NIC	435.115	3	145.038	1.800	.159	.096	.441
Error	4110.396	51	80.596				
Total	11742.834	56					
Corrected Total	4733.884	55					

**Table 69. ANCOVA of Center Time, Female No Stress, T2 – Experiment 1&2**

ANCOVA of Center Time, T2- Experiment 1&amp;2

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	97.555 <sup>a</sup>	4	24.389	.488	.745	.037	.156
Intercept	1449.848	1	1449.848	29.006	.000	.363	1.000
BLRt	77.346	1	77.346	1.547	.219	.029	.231
NIC	18.387	3	6.129	.123	.946	.007	.071
Error	2549.247	51	49.985				
Total	11372.587	56					
Corrected Total	2646.802	55					

**Table 70. ANCOVA of Center Time, Female No Stress, T3 – Experiment 1&2**

ANCOVA of Center Time, T3- Experiment 1&amp;2

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	395.869 <sup>a</sup>	4	98.967	1.524	.209	.107	.438
Intercept	3349.519	1	3349.519	51.566	.000	.503	1.000
BLRt	30.825	1	30.825	.475	.494	.009	.104
NIC	381.265	3	127.088	1.957	.132	.103	.475
Error	3312.743	51	64.956				
Total	15436.899	56					
Corrected Total	3708.612	55					

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